THE BALANCE OF FUNDAMENTAL AND APPLIED RESEARCH

THE ninth annual report of the (U.S.) National Science Foundation (see p. 362 of this issue) is the first to appear since the appointment of Lord Hailsham as Minister for Science; and although there is no institution in the United Kingdom which corresponds to the Foundation, it may be taken for granted that Lord Hailsham is likely to consider very carefully activities on which, in two years, the United States has increased its expenditure from some 40 million dollars to nearly 140 million dollars. Some of this work is parallel with that of the Department of Scientific and Industrial Research in Great Britain; but the latter's expenditure of £500,000 in research grants and approaching £1 million on studentships and fellowships looks small against the National Science Foundation's awards of some 46 million dollars in grants for fundamental research, with nearly 16 million dollars more for research facilities and 13 million dollars in graduate fellowships. Moreover, it is clear from the foreword of its chairman, Dr. Detlev Bronk, that the Foundation is tending to assume functions that the University Grants Committee discharges in Britain and that, like that Committee, it is succeeding notably in strengthening the universities in their true functions of research and teaching without encroaching on their freedom.

Nor is this the only contribution to education which the National Science Foundation is making. It was already concerned with the quality and the extent of science teaching in the schools and in the universities of the United States before the National Defense Education Act of 1958 widened its responsibilities in this field, and in three years its expenditure for such purposes has more than trebled. On a much greater scale than the Department of Scientific and Industrial Research in Britain, it is concerned with the dissemination of scientific information, and the current report attests not so much the magnitude of the resources which are made available for such purposes in the United States as the vision and vigour with which these purposes are pursued by the National Science Foundation.

Probably to the British reader, however, the most suggestive part of the report is that which, following the stimulating statement of the director, Dr. A. T. Waterman, surveys the re-appraisal and re-organization of the status of science in the United States during 1958–59. In that section, as in Dr. Waterman's statement, the essential factors which determine the resources available for research and development and condition their deployment are shown, and it is possible to distinguish how far the institutions devised for one particular set of national circumstances react on those performing other functions. The National Science Foundation is an institution peculiar to the United States, and the fact that it performs certain functions, such as the award of fellowships or research grants or the administration of science fellowships for the North Atlantic Treaty Organization which in Britain are executed by the Department of Scientific and Industrial Research or the University Grants Committee, does not imply that either of these bodies should necessarily be modified on the model of the National Science Foundation. It does, however, suggest that the comparative scale or scope of operations might be studied.

Dr. Bronk stresses particularly the way in which, during the past ten years, the support of science has become generally recognized in the United States as a proper and necessary function of the Federal Government. This is attested by the increase, in that period, of the budget of the National Science Foundation from 3·5 to 155 million dollars, while at the same time the universities have been receiving more assistance than ever from individuals, private foundations and industry. If Dr. Bronk's assumption that the Foundation has stimulated this increased and more widespread support of science is sound, it may be worth while for the Minister for Science in Great Britain to consider rather closely exactly how the Foundation functions, more particularly since it is incontestable that the Foundation has notably succeeded in strengthening American universities without in any way encroaching on their prerogatives or deflecting their policies.

Dr. A. T. Waterman's statement, however, is designed to discourage any facile optimism. Science, he observes, is increasingly an instrument of both national and international policy, and for all that has been done, there is still in the United States an alarming dearth of trained teachers, especially for secondary schools, and no prospect that the requisite number will be forthcoming. Indeed, he suggests that the United States has not yet come to grips with the major problem; and his further comment that the cultivation and staffing of a professional group can be realized adequately only if the career is a rewarding one from the point of view of salary, prospects and prestige, as well as the deeper satisfactions that go with the opportunity to serve, is one that bears vitally on the recommendations of the Crowther Report. It is above all the supply of trained teachers that determines the practicability of raising the school-leaving age, and when full weight is given to the argument of the Crowther Committee in regard to timing, the urgency and priority lie on increasing the number of teachers.

Dr. Waterman makes a further comment on the man-power situation. In spite of the surveys made by the Advisory Council on Scientific Policy's Standing Committee on Scientific Man-power, the studies of the National Science Foundation provide the United States with considerably fuller information about the situation in the United States than is available about the position in Great Britain. About

a quarter of American scientists and engineers are engaged in research and development, and Dr. Waterman points out that this pool of talent is the limiting factor in the national research and development effort, and its competence determines the effectiveness of the nation's undertakings. Its size and quality, moreover, are only increased or improved as a result of long-term operations, and there are definite limits to the extent to which the special skills and the fields that will be important in the future can be forecast.

This last is a factor to be weighed when considering whether or not to embark on new fields such as that of space research, and Dr. Waterman is emphatic as to the need for carefully selecting the fields of research, particularly in applied science, in which even a nation with such immense resources as those of the United States can wisely engage. He regards the establishment of priorities, particularly in fields involving highly expensive equipment or installations, as fundamentally a matter for Government; and it was for this purpose that the Federal Council on Science and Technology was recently established. The functions of that body correspond broadly with those of the Advisory Council on Scientific Policy in Britain, and it should be a first objective of the Minister for Science to see that the Advisory Council is effectively placed to co-ordinate the interests and responsibilities of the various Government bodies with research and development programmes, to identify research needs and to advise him on the more effective utilization of our scientific and technological resources and the planning and administration of the programmes needed.

What stands out particularly from the report of the National Science Foundation is the way in which the educational and research aspects are interlocked and co-ordinated policies are now being concerted. Dr. Waterman, for example, advocates a man-power policy which endeavours to identify talented students in order to ensure that those with aptitude for science and engineering receive the appropriate professional training, more especially in fundamental science. The close relation between teaching and research leads him to emphasize the economic limits on research, and the way in which fundamental research, on which the United States is spending only some 8 per cent of its total expenditure of 10,000 million dollars on research and development, is the main limiting factor on development. Dr. Waterman makes the further observation, which also Lord Hailsham should note, that failure to provide full support may seriously impair the effectiveness of a project.

Dr. Waterman's main argument is for more generous support of fundamental research in the United States and for a more careful scrutiny of the effort devoted to applied research. The question of priorities is equally important in Britain, though it may well be that it is the effort in applied research that should be increased. Be that as it may, it is as essential as in the United States that the programme that is evolved should be supported by an informed body of public opinion, and that the resources placed at its disposal should be adequate in the first instance and not liable to be curtailed from year to year.

This will become the more important if there is any considerable extension of the system of research institutes, to which Sir Harry Melville referred in an address on scientific research in British universities given at Newcastle upon Tyne on September 22. At present the system is limited to nuclear physics, where the Department of Scientific and Industrial Research has been financing in some six universities the construction and largely the maintenance of particle accelerators which are now housed, maintained and operated in a central institute or institutes for the benefit of more than one university, so as to facilitate economic operation, as has been done at the European Organization for Nuclear Research in Geneva. Similarly, the National Institute for Research in Nuclear Science, where a proton synchrotron of an energy of 7 GeV. is under construction, has not a wholly permanent staff, and its work derives inspiration from the university staff who work there.

Much has still to be done to work out the most satisfactory arrangements for such institutes, but Sir Harry Melville stated that about 27 per cent of university expenditure on research in the United States is used in this way, and that experience has established definite benefit to the universities from such an association. The mounting costs of research equipment may well encourage similar developments in other fields, and it is as desirable that the implications, administrative as well as financial, of such a system of special institutes in association with the universities should be fully examined before some urgent problem leads to the establishment of procedures that are unsound. The report "Strengthening American Science", issued in December 1958 by the President's Science Advisory Committee (see *Nature*, **183**, 1215; 1959) showed that this and other problems of financing university research and the relations between Government and universities are receiving close attention in the United States by minds as sensitive to the essential issues as are the University Grants Committee and the Advisory Council on Scientific Policy in Britain.

On the balance of scientific research, the National Science Foundation indicates some important developments. The Foundation has now assumed responsibility for research on the modification of weather, and in March 1959 announced grants for this purpose totalling 1·13 million dollars. At the same time it announced the establishment of an Antarctic Research Programme to continue scientific activity based largely on certain programmes of the International Geophysical Year. There have also been calls from the scientific community for increased effort in particular critical fields. For example, a group on medical research has urged systematic increase in Federal support for medical research from 330 million dollars in 1957 to about 900 million dollars in 1970, with at least a doubling of the number of research workers. A Committee on Oceanography has recommended doubling the support of fundamental research in the marine sciences during the

next ten years at a cost of a further 650 million dollars, about half of this coming through the National Science Foundation. A report on atmospheric research envisaged an institute established and operated by a university committee and financed through the National Science Foundation at a capital and operating cost over six years of some 70 million dollars, as well as further encouragement and support of research in atmospheric physics in existing university departments. President Eisenhower, moreover, has already recommended the financing by the Federal Government of the construction of a large new electron linear accelerator over six years at a cost of some 100 million dollars. The statement also recommended further support of research in high-energy physics by the Atomic Energy Commission, the National Science Foundation and the Department of Defense, and that support for the construction and operation of high-energy accelerators should be increased to 135 million dollars by 1963.

It is not only the scale of effort in the United States that is changing. The Federal organization to meet the scientific needs of the nation is more fluid, and proposals have been made for the creation of departments of science and technology or of science, and for a commission to examine such proposals. It appears, however, that such measures as the establishment of the Federal Council for Science and Technology and the appointment of Dr. W. R. Brode as science advisor to the Secretary of State, following the earlier appointment of a Special Assistant for Science and Technology to the President himself, have largely provided what is needed to support the National Science Foundation, and these arrangements should be given full opportunity to function before decisions are taken as to the need for, or value of, some further centralized agency. Dr. Brode has already expressed deep concern with these questions of organization, but while he is obviously not entirely satisfied that the recent changes provide all that the United States needs, he seems disposed to advocate further study of the organization of science and scientific policy rather than immediate action.

The two points in the work of the National Science Foundation that appear to deserve most careful attention in Britain are the work being done to extend and improve the quality of education in science and technology, and the means by which the balance of research is fostered or varied. On the first, it is not the scale of educational effort that should be noted: Sir Harry Melville has forecast expenditure by the Department of Scientific and Industrial Research on studentships and fellowships of £4 million or more in a few years, probably on some 3,000 awards, or 30–40 per cent of the present total number of research students in the universities, with a further £1–2 million on research grants. It is rather the other measures adopted by the Foundation to assist teachers of science and technology to improve their standard of teaching, to assist the schools to improve their equipment and particularly to make the studies of new techniques and methods of presentation, which could make even the existing supply of teachers more effective.

Here very little has yet been done. The National Science Foundation obviously has a considerable advantage in being able to undertake such studies side by side with its efforts to assist students to take up courses in science and technology and to undertake research; but the Minister for Science is well able to deal with the situation on appropriate lines in Britain. He has the authority to secure whatever co-ordination may be desirable between the work of the Agricultural Research Council, the Medical Research Council, the Overseas Research Council and the Department of Scientific and Industrial Research in such matters as the award of research grants, fellowships or studentships. Further, he would seem to be the most appropriate person to explore the possibility of increasing co-operation with industry and elsewhere.

Very great stress was laid on this by Sir Harry Melville from two points of view. First, Sir Harry emphasized the importance of increasing support for the universities of Britain from other sources so as to prevent their dependence on financial support from the State becoming overwhelming. At the same time, he is convinced that in carrying the main responsibility for fundamental research, the universities should also be responsible for seeing to its balance and the closing of any gaps that may occur. Secondly, he urged that multiple sources of support provided an insurance against mistaken decisions and the refusal of support to a worthy project. The soundness of that view is to be seen to some extent in the existence of differing opinions as to the importance and value of space research, and it might become more important as efforts are made to extend the amount of applied research undertaken in Britain.

Mr. Aubrey Jones, former Minister of Supply, has frankly advocated the establishment of a Minister for Technology rather than a Minister for Science, and rests his argument largely on the prospective reduction of defence expenditure relative to national income. He does not believe that, with defence expenditure little more than 7 per cent of the national income and the defence scientific effort approaching 50 per cent of the total scientific effort of Britain, the rate of technological advance is likely to be maintained. While he thinks that the economic burden of armaments is exaggerated, he suggests that a prime responsibility of the Minister for Science is to find a substitute for the technological role taken by armaments in a free society.

If this contention is sound, clearly the Defence Research Policy Committee and the Advisory Committee on Scientific Policy may require to work much more closely together. Here again it would seem that the Minister for Science is best placed to supply the necessary guidance and to deal with those factors, such as excessive secrecy and departmentation, which may hinder the free exchange of views and information. The problem of communica-

tion indeed requires to be attacked with far more energy, vision and urgency than is apparent in recent references to it in reports from the Advisory Council. There is indeed much to be done to prepare the way for the difficult decisions that cannot long be postponed regarding the balance of research effort, both fundamental and applied, in Britain; and the deployment of our trained man-power in defence, industry, teaching and elsewhere, and the measures to be taken to ensure that the supply is adequate in quality and in numbers. The National Science Foundation's report, although prepared mainly for American readers, indicates numerous points calling for fresh thought and even inquiry in Britain, and it is to be hoped that it will be carefully studied by the Minister for Science and his advisors, the Parliamentary and Scientific Committee and professional associations of scientists and technologists, who are able to assist in forming that intelligent public opinion required to sustain public policy.

APPLIED RADIATION CHEMISTRY

Chemische Reaktionen Ionisierender Strahlen (Radiation Chemistry)

Herausgegeben von Prof. Hermann Mohler. Pp. 299. (Aarau und Frankfurt am Main: Verlag H. R. Sauerländer und Co., 1958.) 30.80 D.M.

A SIGNIFICANT diminution in the cost of atomic power could be achieved if the energy at present 'lost' as radiation were used to achieve large-scale chemical production or processing. In many countries, private and government laboratories exist to explore the possibilities of applied radiation chemistry, and already a number of processes have been, or are being, used commercially. Hitherto the use of radiation in the chemical industry has been very small, primarily because radiation merely generates in chemical systems reactive species such as ions and/or free radicals which in most cases can be produced in a more controlled manner by more conventional chemical means. This fact and certain inherent disadvantages in the use of radiation are likely to limit its successful application to fields where chemical methods are either non-existent or very difficult to apply. The book under review has been written in the belief that the time has now come when the chemical technologist should know something of the principles and applications of radiation chemistry.

The book originated in a course of lectures in the University of Basle in 1956, and some of the authors contributing to this book took part as lecturers. After a brief definition of the subject, the first half of the book is concerned with a discussion of the necessary background knowledge of radioactivity, followed by an account of radiation sources (parts of which read like a technical sales brochure) and dosimetry, with a minor chemical digression (pages 54–68) in which a sketchy account of some radiation chemical mechanisms is given. The latter part of the book deals with the interaction of radiation and matter and presents somewhat cursory accounts of selected radiation-induced reactions in solid, liquid and gaseous phases, and concludes with two chapters, rather surprisingly in English, entitled "The Effects of Atomic Radiation on Polymers" and "Radiation-induced Reactions of Potential Industrial Importance".

The book displays several of the disadvantages of composite authorship. For example, in the whole of the last chapter the phrase 'radiochemical reaction' is given a meaning quite different from that commonly accepted and from that carefully specified by the editor and writer of the introduction. Again, there is a great unevenness in level of treatment; the chapter on polymers, though by an expert, is almost at the level of popular science exposition, whereas that on dosimetry is a good deal more profound. These defects are minor in comparison with several distorted and often erroneous notions about radiation chemistry which the book contains. The stated origin of the hydroxyl radicals in irradiated water (p. 63), the mechanism of formation of H_3O^+ on electron impact on water vapour (Table 4·08, p. 194), and the mechanism of the reduction of ceric ions (p. 152), all contain inexcusable errors which are not printing slips. The complete omission of ion-molecule reactions and the inadequate treatment of the competition between diffusion and combination of radicals, both matters of central importance, are serious deficiencies. When one reads phrases such as the following about the oxidation of sulphur dioxide by oxygen in aqueous solution, "Attention will have to be given to the effect of trace metals, for example, iron and copper, on the reaction" (p. 251), or "There is no published information to indicate to what extent chain reactions play a part" (p. 249), one marvels how the author of this chapter can have overlooked the classical works of Bäckström and others. Any chemist or chemical technologist using the Fricke dosimeter to determine doses or dose-rate who uses the G values for this system given on pages 156 and 161 must be content with values which will be 6–7 per cent too high.

Apart from the dropping of an 'h' from Shrivenham, the book is well printed and produced.

F. S. Dainton

THE THEORY OF DAMS

The Theory of Storage

By Prof. P. A. P. Moran. (Methuen's Monographs on Applied Probability and Statistics.) Pp. 111. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1959.) 13*s.* 6*d.* net.

MANY of the individual problems of applied probability have a very respectable antiquity; thus queueing theory began with Erlang in 1908, what we now call birth-and-death processes were first studied by Galton and Watson in 1873, and the use of probabilistic models in physics and astronomy can be traced back to much earlier dates than these. But it was not (in Great Britain at least) until just after the Second World War that the full range of possibilities was widely recognized; during the past fifteen years the subject of applied probability has grown to such an extent that it now rivals in importance its elder sister, the statistics of experimentation. That the publishers of this new series of monographs recognize this fact is clearly shown by the general title which they have given to the series, by the individual titles of the five volumes so far announced (only one of these is concerned with statistics as ordinarily

understood), and by their happy choice of Prof. M. S. Bartlett as general editor of the series.

In the monograph reviewed here Prof. P. A. P. Moran of the Australian National University, Canberra, writes on the theory of storage systems in which either the input or the output is of stochastic character. Most space is given to problems of the first type ("The Theory of Dams"); here a random input of water feeds a reservoir or a system of reservoirs subject to overflow and release rules of various types, and one is interested both in the conditions for statistical equilibrium and the statistical distributions which characterize it, and also in transient phenomena such as the risk that the reservoir will run dry within a given time period when the initial conditions are known. This is a very recent branch of applied probability, and can be said to be Prof. Moran's peculiar creation, for apart from some exceedingly interesting pioneer work by Hurst practically all the mathematical papers devoted to the subject have been written by Moran himself or by those influenced by him. The programme of research opened up by Moran in 1954 is still very far from complete; much more remains to be done on systems with a (random) continuous input, very little is known yet about the behaviour of networks of reservoirs, and a satisfactory treatment of correlated inputs is still lacking (as it is also in queueing theory, although recent work by Dagnall, Winsten, and Wishart has shown that significant progress can be made if the problem is suitably formulated). Those familiar with the earlier literature will be very grateful to the author for this timely survey of what has been done and of what remains to be done; others will be equally grateful for the possibility which this book offers them of joining in a very active programme of research.

D. G. KENDALL

QUANTUM EFFECTS ON A MACROSCOPIC SCALE

Liquid Helium

By Prof. K. R. Atkins. (Cambridge Monographs on Physics.) Pp. x+312. (Cambridge: At the University Press, 1959.) 60*s*. net.

THE strange behaviour of liquid helium at low temperatures has been the subject of much work and thinking for some time. Ever since Kamerlingh Onnes succeeded in liquefying helium some fifty years ago, work has been going on with this single substance; more and more new phenomena have been discovered and a new field of experiments and theory has been established. At first, the difficult techniques for obtaining the necessary low temperatures meant that the work was concentrated in a few laboratories which became famous in this field. With the more recent methods these low temperatures are easier to obtain, and much work is going on in many places all over the world. The subject is still in rapid development. A new monograph which tries to select the essential features in such a vast field of experiments and theoretical thinking is very welcome. At the same time, it is a somewhat bold enterprise to write a monograph on such a rapidly progressing subject. A few points were already out of date by the time that the book was printed.

The wide variety of strange phenomena connected with liquid helium led slowly towards a pattern of basic facts. Some of these are by now essentially understood, others are not, and many more are perhaps still to be discovered. Theoretical ideas and experimental discoveries proceeded hand in hand in a fruitful co-operation. There are many striking examples of this interaction between theory and experiment. The concept of elementary excitations which move around in the superfluid background was introduced by Landau and is essential for the understanding of many properties of liquid helium. These excitations, phonons and rotons, characterize the low-lying excited states of the system. Landau's predictions about the excitation spectrum have been recently confirmed by neutron-scattering experiments. The elementary excitations form the 'normal component' of the liquid, which together with the superfluid component leads to a 'two-fluid' theory from which many features of the hydrodynamic and thermal behaviour of liquid helium can be understood. Other features which are less well understood are related to the formation of vortices. According to the ideas of Onsager and Feynman, the strength of these vortices has quantized values, a prediction which has found experimental verification in the past few years.

Quantum properties play an important part in the whole behaviour of liquid helium, and some manifestations of these are on a macroscopic scale. The Bose statistics obeyed by the helium-4 atoms seems also to be essential for the superfluid behaviour of helium. The helium-3 isotope which obeys Fermi statistics does not show superfluidity. Though many features of liquid helium are understood, much progress is still needed in a detailed theoretical description. The phenomena are related to the collective behaviour of a large number of particles, and the wave mechanics of such a system is of considerable mathematical complexity. But a better understanding of the collective behaviour of helium atoms may be of significance also in connexion with other similar problems of physics.

The book under review succeeds in a fair selection of the relevant experimental facts in making a good systematic treatment. This will be of value both to the expert and for reference. Some parts in the theoretical sections are perhaps not of the same standard, and especially the author's estimate of the theoretical achievements seems to be slightly overoptimistic. The book covers a lot of ground, it makes pleasant reading, and will be enjoyed not only by the specialists but also by those who look merely for orientation in this rather exciting subject.

J. G. VALATIN

FRESHWATER INVERTEBRATES

A Guide to Freshwater Invertebrate Animals

By Dr. T. T. Macan. Pp. x+118. (London: Longmans, Green and Co., Ltd., 1959.) 11*s*. 6*d*. net.

THE importance of freshwater biology for scientific, economic and educational purposes has greatly increased in recent years and the many biologists, both amateur and professional, now interested in the subject, will be grateful to T. T. Macan for writing an attractive, straightforward and well-illustrated "Guide to Freshwater Invertebrate Animals".

The line drawings, of which there are more than two hundred, are simple, clear and attractively

finished and make up for some of the carelessness in the choice of words. To anyone unfamiliar with the conventions applied in describing mollusc shells the definition of a sinistral shell will mean very little: "if it be held upright with the aperture towards the observer the aperture is to the observer's left". Readers requiring a definition of sinistral in this context are the very ones least likely to know the meaning of 'upright'. Fortunately most of the ambiguities and other obscurities can be resolved by reference to a figure or to an opposing couplet in the key; but this takes a little time, and the distractions they evoke are further aggravated by expressions like "Swimming quick" which may be a grammatically appropriate antithesis to "Swimming laboured", but could easily have been avoided.

Apart from these minor blemishes the "Guide" is a pleasure to use. The practice of giving the dimensions of the specimens figured in millimetres rather than giving the magnifications is commendable and will save much mental arithmetic. It is, however, unfair to expect all the readers for whom the book is intended to know that the length of an insect, for example, does not include in its measurement any appendages projecting at either end.

Most authors of taxonomic keys become slaves to the system, and for the sake of uniformity attempt to find simple characters to separate large groups which are too complex for this to be done satisfactorily. It is for this reason that most keys require the reader to inquire into the most obscure and insignificant details, particularly in the early stages of the key, where the groups to be separated are the most complex. This is very discouraging until the student learns by experience how to bypass the difficulty by recognizing the major groups by sight rather than by resolving the alternatives presented in the key. Dr. Macan avoids this trouble in a delightfully simple way: where to persist in the format of a key would lead to the use of impracticable alternative questions he abandons the format and substitutes a straightforward explanatory paragraph. By way of compensation, perhaps, the author makes use of the key format for his references to further literature. The number of species of freshwater invertebrates in Great Britain is far too great for them all to be dealt with in a single work, so in many places the key inevitably ends in a family or other group name rather than a genus or species, and here where the investigator wants it is a reference to further literature on the group. Macan's "Guide" is thus more than a key to the invertebrates, it is also a master key to other keys.

J. P. Harding

DOWSING—FACT OR FICTION?

Water-Witching U.S.A.

By Evon Z. Vogt and Ray Hyman. Pp. xi+248. (Chicago: University of Chicago Press; London: Cambridge University Press, 1959.) 37*s*. 6*d*. net.

THE difficulties of popularizing science are well known; they are probably greatest in fields such as psychology and sociology, where most readers are quite unaccustomed to the possibility of a scientific approach. The book under review shows what can be done even in a field that superficially might seem rather unlikely. In turning to dowsing or 'water witching', the writers, of course, have the advantage that most lay readers will have some interest in the problem and will at least have heard of the claims made for this 'science'. Their account is well written, well documented, critical in the best sense and, above all, intensely readable. It can be recommended to anyone interested in the subject as a model of popularization, and few people will lay down the volume without having learned a few new facts at least from the authors' wide-ranging discussion.

In their book they pass from the reasons for dowsing, through its history, to a consideration of the question of whether it does in fact work or not. As they point out, there is fairly universal agreement that the stricter the controls become which are imposed on the dowser, the less frequently does his score deviate from the chance-level. In this, there is a striking parallel to other sets of beliefs, such as those associated with mediums, clairvoyants, phrenologists, graphologists, and Freudian psychotherapists. The unbiased reader will be unlikely to disagree with their conclusion that there is no scientific basis for water divining, and that recourse to it and belief in it are both based on psychological causes which are independent of reason and scientific proof. Particularly good in this connexion is their discussion of scientific methodology applied to problems of this type; even those least aware of modern psychological methodology could scarcely fail to follow their description step by step of the reasons why controlled experiments are necessary, and the way in which such experiments should be carried out.

The authors go on to discuss psychological theories which might account for the muscle twitches of the dowser, including a discussion of the famous 'talking horses' and other so-called 'parapsychological' phenomena. Here again, the discussion is lucid, well supported by strictly experimental findings, and altogether convincing. Their main point in bringing in these apparently extraneous facts is this. Modern psychology has demonstrated the truth underlying Carpenter's ideo-motor theory, to wit, that a person's thoughts tend to be mirrored in certain small incipient muscle movements or twitches. These can be used as signs or signals by a clever medium. By the same token, they can also, when amplified by the forked twig held by the water diviner, lead to an unconscious indication of the diviner's thoughts about the likelihood of water-bearing properties of a particular piece of ground—notions themselves in many cases derived from his personal familiarity with the neighbourhood, or even semi-scientific knowledge of geological indications.

The book ends with some chapters on the kind of people who undertake dowsing, the kind of people who employ dowsers, and the reasons why they do so. The facts discussed there are derived from the United States only, where dowsing appears to be much more prevalent than in Britain. The interested reader might like to consider whether the descriptions furnished by Vogt and Hyman of the typical dowser and his employer fit in with national stereotypes about the United States and Britain; it would certainly be interesting to have some similar facts about dowsing in the latter. Even without this, however, most readers will find this an interesting, enjoyable and well-written book, which will tell them all they need to know about 'water witching'.

H. J. Eysenck

British Industrialists

Steel and Hosiery, 1850–1950. By Charlotte Erickson. (The National Institute of Economic and Social Research. Economic and Social Studies, Vol. 18.) Pp. xxi+276. (Cambridge: At the University Press, 1959.) 40*s*. net.

THIS book gives the results of a survey of the social origins and careers of about 1,000 managing partners and executive directors in the hosiery industry of the Nottingham area and in the heavy-steel industry during the past one hundred years. It is thus in the first place a historical study, but the work was planned on the assumption that important differences might exist in the recruitment of management by different industries, depending upon their age, traditions, structure and organization. It does not claim more than to present some empirical data on the social origins of business leaders and on the use made of the natural ability of able individuals, and makes no attempt to construct principles for the selection of leaders.

Nevertheless, as Prof. T. S. Ashton points out in his foreword, this admirably produced volume contains material highly relevant to present issues. It gives some background for present-day thinking about the selection of leaders and about training for management that should be carefully weighed in planning the expansion of higher technological education at the least. These studies bear closely on such questions as what constitutes the best education and experience for business leadership, the relative place of education and of experience, the appropriate age of appointment of directors, as well as retirement. Such questions cannot be dealt with in averages without regard to the particular individual's rate of development and retention of powers, but they need consideration, and this book demonstrates the practicability of obtaining evidence and shows how it can be obtained. Moreover, the two industries actually chosen offer the possibility of some tentative inferences about the growth of the impersonal corporation upon the selection of business leaders in Great Britain. Indeed the book may well gain in value as further studies on such lines are made in other industries and the results become available for comparison.

R. BRIGHTMAN

The Study of Man

The Lindsay Memorial Lectures, 1958. By Prof. Michael Polanyi. Pp. 102. (London: Routledge and Kegan Paul, Ltd., 1959.) 7*s*. 6*d*. net.

THESE lectures form a most valuable companion to the author's "Personal Knowledge", in which he goes far towards rejecting the objectivity of science in that, as a discipline, it is commonly thought of as detached and impersonal. An individual, even passionate, element is essential. The three discourses are entitled (1) "Understanding Ourselves", (2) "The Calling of Man", (3) "Understanding History". This emphasis upon understanding rather than upon prediction (the usual goal in theoretical studies) marks the writer as a true epistemologist. The importance of such an outlook to-day lies in its capacity to offset at least some of the evils of specialization without becoming merely dilettante in the process.

The author is now ready to do something well worth the attempt, namely, to transpose much of Gestalt-psychology into a theory of knowledge. Why this has not happened before is largely because its devotees were too passive, and inclined to under-estimate the personal element. In this, and in many other ways, Prof. Polanyi shows himself an artist, not least when he insists that those to whom the internal splendours of mathematics are a closed book have no knowledge of the subject.

It remains to see all this in the light of the late Lord Lindsay's outlook on education, and his early efforts on behalf of the University College of North Staffordshire in particular. He would have delighted in the liberalism which makes the act of knowing an urge to service.

F. I. G. RAWLINS

Russian–English Glossary of Solid State Physics

Edited by I. Emin. Pp. iii+90. (New York: Consultants Bureau, Inc., 1958.) 10 dollars.

Russian–English Glossary of Optics and Spectroscopy

Pp. iv+78. (New York: Interlanguage Dictionaries Publishing Corporation, 1959.) 10 dollars.

THESE two glossaries are of about the same size, the same format and edited by the same editor, but are published by different organizations. They represent very useful compilations of Russian terms belonging to the respective branches of physics, but although each glossary contains some 4,000 terms the user of these glossaries, as of any other specialized technical compilation, will often find certain terms missing. This applies to all specialized glossaries for the obvious reason that there are no hard and fast separations between specialized and general terms in science. Thus, for example, a very important Russian term of a French derivation 'etalon', meaning 'standard', such as 'standard sample' or 'standard measure', also the term 'brus' or 'brusok' meaning 'bar', or again names of certain chemical compounds or common minerals, are all missing from these compilations. The price of each of these glossaries is rather high.

S. I. TOMKEIEFF

Outline of Human Genetics

By Prof. L. S. Penrose. (Contemporary Science Series.) Pp. xii+146. (London: William Heinemann, Ltd., 1959.) 12*s*. 6*d*. net.

THIS is an excellent and lucidly written introduction to human genetics for the general reader. As one would expect, the treatment is well balanced and the factual basis correct and up to date. Of particular interest is the last chapter ("Eugenics and Dysgenics"), in which the author, himself the Galton professor of eugenics in the University of London, gives many good reasons for a conservative attitude towards practical measures in this field.

H. GRÜNEBERG

Die Cactaceae

Handbuch der Kakteenkunde. Von Curt Backeberg. Band 3: Cereoideae (Austrocactinae). Pp. iii+1361–1926+tafel 108–160. (Jena: Gustav Fischer Verlag, 1959.) 85 D.M.

THIS further volume of Herr Backeberg's colossal new handbook to the Cactaceae (*Nature*, 184, 1010; 1959) continues the account of the subfamily Cereoideae as far as the end of the 'sippe' Austro-echinocacti of the subtribe Austrocactinae. Among the larger genera included are *Lobivia* (72 species), *Gymnocalycium* (58) and *Parodia* (36). As before, the volume is copiously illustrated.

A BASIS FOR A GENERAL SYSTEM OF INSECT MIGRATION AND DISPERSAL BY FLIGHT

By C. G. JOHNSON
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WHENEVER the start of a 'mass migration' of insects has been described—and this is very rare in spite of a voluminous literature—the insects have nearly always been making either their first flight as new adults or one very soon afterwards. This fact is usually mentioned only incidentally and has been observed intentionally in very few insects[1–15] : it has never been brought forward as a general principle, though it may provide a central feature for a long-needed, general system of insect migration and dispersal.

Studies of insect migration have usually been preoccupied with special qualities of the flight such as persistence, long distances travelled, collective flight or apparent control over the direction of displacement ; and migration is usually defined in these terms[1–15]. But no matter how spectacular these behavioural qualities may be, they are so variable that definitions based on them as to what is or is not 'migration' or what is 'active' migration and what is 'passive' dispersal, become arbitrary, controversial and unsatisfactory when applied to all the types of insect which are said to migrate.

I suggest that the only common feature which is obvious at present in the mass migrations of insects in general is the active exodus flight by new adults from the breeding site followed by one or more flights, varying in character from species to species, to a new breeding site. This is what I shall mean by migration. I shall take mass migrations as the starting point for analysis.

Records of Post-teneral Migration Flights

I have so far found no exceptions to my conclusion that mass migrations begin in the first place either as first flights at the end of the teneral period or soon afterwards.

The examples supporting the conclusion are given in Table 1 and refer to recognized migrants like *Vanessa cardui*, *Ascia monuste*, locusts and dragonflies and to less spectacular ones ; to organized drifters like aphids, and to Isoptera and crepuscular or nocturnal chironomids, which normally fly a very short distance but which can also be said to migrate in the above sense of the word. These examples include insects of several orders and cover a wide range of flight speed, duration and orientation.

The teneral period is taken here as beginning at emergence from the last immature instar and ending at full flight-worthiness, although pigmentation may be incomplete as in dragonflies. It varies inherently from about 1 hr. (*Vanessa cardui*)[16,17] to several days (locusts)[4,18,19] ; its length is affected by temperature, as with any developmental process[20].

Perspectives in Migration Studies in the Light of the Post-teneral Flight

A recognition of the principle of post-teneral exodus flight brings new order and interesting speculation to the subject.

(1) *Periodicity of the first flight.* The daily synchronization of mass flights is not primarily behavioural, as with birds. Except in ants and termites it is caused by rhythms of emergence from the last immature instar, and by variation in the teneral period with changing temperature, in association with light and temperature thresholds for take-off[8–12]. Flight peaks may be repeated daily or within the day : total numbers migrating each day follow the curve for seasonal alate population change[47]. Seasonal synchronization may be caused by breaking of diapause[48]. Such periodicities are fundamental to the mass migration process.

This synchronized flight by individuals without apparent effect on each other is often called 'swarming'. This term is also used to describe aggregations by mutual attraction, which is quite a different process.

(2) *Possibility of teneral or post-teneral 'conditioning'.* The initial orientation of the insect in flight is probably 'imprinted' during the teneral period, or determined at take-off. The analogy with flight of bees comes to mind. Distances flown (within 24 hr.)

Table 1. Examples of Exodus Flight Immediately at the End of the Teneral Period
Recognized migration asterisked

Exopterygota
- Odonata
  - Of general occurrence (refs. 21, 22)
  - * *Macrogomphus annulatus* (Selys) (Gomphidae) (ref. 23)
  - * *Anormogomphus kiritschenkoi* (Bartenef) (ref. 23)
  - *Gomphus vulgatissimus* (L.)
  - * *Lindenia tetraphylla* (Van der Lind.) (ref. 23)
  - *Anax imperator* Leach (Aeshnidae) (ref. 15)
  - * *Anax guttatus* (Burm.) (Aeshnidae) (ref. 23)
  - *Anax junius* Brauer (Aeshnidae) (ref. 28)
  - *Aeshna mixta* Latr. (Aeshnidae) (ref. 24).
  - * *Hemianax ephippiger* (Burm.) (Aeshnidae) (ref. 23)
  - * *Orthetrum trinacria* (Selys) (Libellulidae) (ref. 23)
  - * *Sympetrum striolatum* Charp. (Libellulidae) (ref. 25)
  - * *Sympetrum fonscolombii* (Selys) (Libellulidae) (ref. 24)
  - * *Pantala flavescens* Fabr. (ref. 23)
  - * *Lestes malabarica* Fraser (Lestidae) (ref. 23)
- Orthoptera
  - * *Schistocerca gregaria* Forsk. (Acrididae) (refs. 4, 19)
- Isoptera
  - All species (ref. 26).
  - *Drepanotermes silvestri* Hill (ref. 27)
  - *Hamitermes perplexus* Hill (ref. 27)
- Hemiptera
  - * *Blissus doriae* Ferr. (Het : Lygaeidae) (ref. 29)
  - * *Dysdercus sidae* Montr. (Het : Pyrrhocoridae) (ref. 30)
  - * *Aphis fabae* Scop. (Hom : Aphididae) (refs. 6, 8, 9, 31)

Endopterygota
- Lepidoptera
  - * *Calopsilia crocale* Ca. (Pieridae) (ref. 32)
  - * *Calopsilia pyranthe* L. (Pieridae) (ref. 33)
  - * *Ascia monuste* L. (Pieridae) (refs. 13, 14, 34)
  - * *Vanessa cardui* L. (Nymphalidae) (refs. 16, 17)
  - *Vanessa californica* Bdv. (Nymphalidae) (ref. 35)
  - * *Loxostege sticticalis* L. (Pyraustidae) (ref. 36)
  - *Hemileuca oliviae* Ckll (Lasiocampidae) (ref. 37)
  - There are very many references to migrations by *apparently* newly emerged butterflies and moths (ref. 1)
- Diptera
  - * *Aedes taeniorhyncus* (Wied.) (Culicidae) (refs. 10–13)
  - *Chironomus brevibucca* (Kieff.) (Chironomidae) (ref. 38)
  - There are many references suggesting post-teneral exodus flights in mosquitoes (ref. 39)
- Hymenoptera
  - *Diprion polytomum* (Htg.) ? (Diprionidae) (ref. 40)
  - Generally in ants (ref. 41)
- Coleoptera
  - Several *Dendroctonus* spp. (Curculionidae) (ref. 42)
  - * *Anthonomus grandis* Boh. (Curculionidae) (ref. 43)
  - *Euthyrrhinus meditabundus* Fabr. (Curculionidae) (ref. 44)
  - * *Diabrotica duodecimpunctata* Fabr. (Chrysomelidae) (ref. 45)
  - *Promecotheca opacicollis* Gestro (ref. 46)

possibly depend on the time of day when flight maturity and take-off occur, that is, on the period remaining before flight is stopped by darkness or falling temperature. Too long an enforced wait before take-off, leading to sexual maturation or fertilization, apparently inhibits migration altogether in *A. monuste*[13] and *Hemileuca oliviae*[37]. Reversal of positive phototaxis with changes in temperature (and humidity)[49–51] may influence take-off behaviour and subsequent flight.

Thus, variation in initial orientation and flight duration, or whether the exodus flight will occur or not, could be expressed collectively according to the daily periodicity.

(3) *Take-off flight.* The individual take-off flight at the exodus appears to be very similar for several insects of different orders. It is often described as a vigorous flight upwards (positive phototaxis)[7,13,30,36,52] or at a steep angle, to about 20 ft. when turbulent transport determines speed and direction or the insects stop climbing and are reported as flying off in the same direction[10–12,15–17,30].

Some species may fly off without delay (aphids, *V. cardui*); others (locusts, *A. monuste*) first make a few, relatively short preliminary flights.

(4) *Duration of migration.* This is partly a species characteristic, probably evolved in relation to survival. Locusts and some Lepidoptera may continue to fly intermittently for many days, covering great distances; others fly for only a few hours (aphids) or even minutes (some Isoptera) before migration ends or flight ceases permanently (Isoptera: some aphids). The exodus flight, however, and sometimes its continuation, appears always to be of relatively great vigour.

(5) *Migratory and other flights.* At present it is not clear if the exodus flight and its more or less immediate continuation are different in quality from other flights which occur later: it is claimed that they are, soundly I think, for Lepidoptera[1–3] and mosquitoes[10–14] at least. But with some insects it is not possible to be certain about this. It seems to me that it is best to make the chronological distinctions first, and that the failure to do this has greatly blurred the migration picture.

(6) *Aggregation.* Insects may aggregate at take-off (locusts) or afterwards (*Danaus plexippus*)[53] or at hibernation or æstivation (for example, Coccinellids). This is a special feature, secondary to synchronization of flight by simultaneous maturation when individuals fly away together but apparently without affecting each other.

(7) *Hibernation and æstivation.* I presume that they are exodus flights which end in hibernation or æstivation, followed later by so-called 'return migration' towards former breeding sites.

(8) *The 'return flight'.* The 'return' flight has been considered a necessity without which a species would lose its breeding sites[3]. This assumes that all the females of the original population transfer to places unsuitable for breeding: this has not been proved. Direction, as well as distance, from the original source vary and migration is dispersive (as compass diagrams show[1–3]); some insects may remain behind[13,37]. The habitat may thus be replenished from within or from directions other than that of the main exodus[54]; there is, however, often a seasonal bias in different directions. The analogy with birds should not be pressed too far.

(9) *Causes of migration.* Current lack of food or space is often said to cause migration, but I postulate that to make the original exodus is the normal individual action of many new flight-mature insects, irrespective of the size of the population, the immediate food supply or space. Shortages of food or space may cause insects to move; such shortages often coincide with a mass exodus, but they are not necessarily cause and effect. Exodus flights are, no doubt, made also by individuals from the smallest possible populations but pass unnoticed. I see migration as an evolved adaptation rather than as a current reaction to adversity.

(10) *The adult as a migrant stage.* The idea that the adult represents a stage the primary function of which is dispersal fits in with my conclusions; for a primary function would be expected to occur as soon as possible once the adult stage is reached, when the insect is fresh.

(11) *Migration and reproduction.* The exodus flight having evolved for recolonization, it therefore precedes oviposition; in fact, in *Aphis fabae*, larviposition is delayed until induced by flight[55], and this may well be the general tendency.

(12) *Migration and dispersal.* The initial exodus flight appears to be generally very active and continuing into the migratory flights lead to dispersal except where they end in aggregations. It remains to find the proportion of the aerial 'plankton' concerned with 'deliberate' post-teneral flights as distinct from being caught up 'accidentally' at other times; but I suspect that post-teneral exodus is the rule with many more species than we realize, though it may be unobstrusive. Insects which do not migrate may well be the exceptional ones.

Conclusions

Mass migratory flights, as commonly recorded, appear to be made primarily by newly emerged adults at the end of the teneral period after moulting from the previous instar and as soon as the insects have full capacity for flight. Commonly, it is the first flight of the new adults, but in some species a number of relatively short flights lead to the exodus flight, which, continuing, becomes dispersive, and takes the insects to new breeding sites, from which new adults of another generation migrate. Exodus flights appear to be made usually by individuals without reference to each other except in special cases (for example, locusts).

This principle does more than repeat that insects leave their breeding places; it says when they migrate, and why they may do so collectively. It distinguishes migration flights chronologically in the first place before attempting to distinguish them behaviourally.

Migration from hibernating or æstivating aggregations of insects also occurs more or less *en masse*, but this is not a general feature; the resemblance to bird migrations (in that a change in behaviour occurs in adults with a previous, different behavioural history) has probably distracted attention from the original post-teneral migration which I postulate is common to all.

The chronology of mass migration now seems more clear: experimental work on migration in many insects might now be planned without waiting for the hitherto unpredictable phenomenon in the field.

This might apply especially to problems of orientation: and to the possible relation of endocrine balance to the active and persistent character of many migratory flights following emergence.

Note added in proof: Since writing this article I have seen P. B. M. Allan's paper (*Entom.*, **75**, 147; 1942) in which he, like Piepers (1890), expresses opinions applying specifically to Lepidoptera, which are similar to the views developed above.

[1] Williams, C. B., "The Migration of Butterflies" (London, 1930).
[2] Williams, C. B., "Ann. Rev. Ent.", **2**, 163 (1957).
[3] Williams, C. B., "Insect Migration" (London, 1958).
[4] Kennedy, J. S., *Phil. Trans. Roy. Soc.*, B, **235**, 163 (1951).
[5] Kennedy, J. S., and Stroyan, L. J., "Ann. Rev. Ent.", **4**, 139 (1959).
[6] Moericke, V., *Z. Angew. Ent.*, **37**, 29 (1955).
[7] Johnson, C. G., *Biol. Rev.*, **29**, 87 (1954).
[8] Johnson, C. G., and Taylor, L. R., *J. Exp. Biol.*, **34**, 209 (1957).
[9] Johnson, C. G., Taylor, L. R., and Haine, E., *Ann. App. Biol.*, **45**, 682 (1957).
[10] Provost, M. W., *Mosquito News*, **12**, 174 (1952).
[11] Provost, M. W., *Mosquito News*, **13**, 196 (1953).
[12] Provost, M. W., *Mosquito News*, **17**, 233 (1957).
[13] Nielsen, E. T., *Bull. Ent. Res.*, **49**, 305 (1958).
[14] Nielsen, E. T., and Nielsen, A. T., *Ent. Medd.*, **26**, 386 (1952).
[15] Corbet, P. S., *J. Anim. Ecol.*, **26**, 1 (1957).
[16] Skertchly, S. B. J., *Nature*, **20**, 266 (1879).
[17] Egli, O., in Loeliger, R., Centre d'observation pour les migrations de papillons, Zurich. Circulaire No. 24, 3 (1950).
[18] Uvarov, B. P., *Bull. Ent. Res.*, **12**, 135 (1921).
[19] Gunn, D. L., *et al.*, *Nature*, **156**, 628 (1945).
[20] Taylor, L. R., *J. Exp. Biol.*, **34**, 189 (1957).
[21] Longfield, C., "The Dragonflies of the British Isles" (London, 1937).
[22] Tillyard, R. J., "The Biology of Dragonflies" (Cambridge University Press, 1917).
[23] Fraser, F. C. (private communication).
[24] Fraser, F. C., *J. Soc. Brit. Ent.*, **2**, 133 (1943).
[25] Fraser, F. C., *Ent. Mon. Mag.*, **81**, 73 (1945).
[26] Ratcliffe, F. N., Gay, F. J., and Greaves, T., "Australian Termites" (Melbourne, 1952).
[27] Hill, G. F., *Bull. Ent. Res.*, **12**, 363 (1922).
[28] Root, F. M., *Canad. Ent.*, **44**, 208 (1912).
[29] Sajo, K., in Webster, F. M., *U.S. Dept. Agric. Bur. Ent., Bull.* No. 69 (1907).
[30] Ballard, E., and Evans, M. G., *Bull. Ent. Res.*, **18**, 45 (1928).
[31] Müller, H. J., and Unger, K., *Züchter*, **22**, 206 (1952).
[32] Piepers, M. C., *Natuurk. Tijdschr. Ned.-Ind.*, **50**, 198 (1890).
[33] Manders, N., *Trans. Ent. Soc. Lond.*, Pt. 4, 701 (1904).
[34] Nielsen, E. T., and Nielsen, A. T., *Amer. Mus. Novit.*, No. 1471 (1956).
[35] Webster, F. M., *Canad. Ent.*, **45**, 117 (1913).
[36] Melnichenko, A. N., *Bull. Plant Protection*, Leningrad, 1st Ser., No. 17, 5 (1936).
[37] Ainslie, C. N., *U.S. Dept. Agric. Bur. Ent., Bull.* No. 85, Pt. 5, 59 (1910).
[38] Fryer, G., *Bull. Ent. Res.*, **50**, 1 (1959).
[39] Horsfall, W. R., "Mosquitoes: Their Bionomics and Relation to Disease" (London, 1955).
[40] Balch, R. E., *J. Econ. Ent.*, **32**, 412 (1939).
[41] Imms, A. D., "A General Textbook of Entomology" (London, 1957).
[42] Hopkins, A. D., *U.S. Dept. Agric. Bur. Ent., Bull.* No. 83, Pt. 1, 169 (1909).
[43] Gaines, J. C., *J. Econ. Ent.*, **25**, 1181 (1932).
[44] Hill, G. F., *Bull. Ent. Res.*, **12**, 63 (1921).
[45] Smith, C. E., and Allen, N., *J. Econ. Ent.*, **25**, 53 (1932).
[46] Kowalski, J., *Ann. Epiphyt.*, **4**, 286 (1917).
[47] Johnson, C. G., *Ann. App. Biol.*, **39**, 525 (1952).
[48] Corbet, P. S., *Nature*, **174**, 655, 777 (1954).
[49] Perttunen, V., *Ann. Ent. Fenn.*, **24**, 12 (1958).
[50] Perttunen, V., *Ann. Ent. Fenn.*, **25**, 65 (1959).
[51] Perttunen, V., and Lahermaa, M., *Ann. Ent. Fenn.*, **24**, 69 (1958).
[52] Peake, F. G. G., *Bull. Ent. Res.*, **43**, 317 (1952).
[53] Lugger, O., *Proc. Ent. Soc. Wash.*, **1**, 256 (1890).
[54] Wiltshire, E. P., *Trans. Roy. Ent. Soc. Lond.*, **96**, 168 (1946).
[55] Johnson, B., *Animal Behaviour*, **6**, 9 (1958).

BINDING OF A HEPATOCARCINOGEN TO THE STRUCTURAL LIPOPROTEIN OF RAT LIVER: ITS BEARING ON THE IMMUNOLOGICAL THEORY OF CANCER

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THE outstanding relevance of specific protein binding by carcinogens to the immunological theory of cancer has been stressed repeatedly since the theory was first proposed[1]. The concept that this specific binding is to a cytoplasmic tissue-specific lipoprotein complex, detectable as tissue-specific antigen, has been steadily developed since that time[2] and there is considerable evidence in its favour.

In hepatocarcinogenesis, central importance has been attached recently to azo-dye binding in the microsomal fraction[3,4]. Since it is known that this fraction, as obtained by differential centrifugation of liver homogenates, includes the cell membrane and ergastoplasm (endoplasmic reticulum + ribonucleic acid granules) it becomes important to decide whether binding of azo-dye to these structures is a factor in the binding found in the fraction as a whole.

Bruemmer and Thomas described a method[5] of obtaining a 'ghost cell' preparation from calf liver and showed it to be an insoluble lipoprotein with a similar chemical composition to an insoluble lipoprotein obtained by Smith *et al.*[6] by differential centrifugation of homogenates. It is thought to represent the structural substance of the cell, that is, the endoplasmic reticulum. Using this method[5], we have obtained a ghost cell preparation from the livers of control rats and rats injected with azo-dye, and confirmed that it is an insoluble lipoprotein. We have also shown, by analysis[7] for bound dye, that firm binding to the protein of this lipoprotein does in fact occur. It is seen (Table 1) that in acute experiments, binding by the hepatocarcinogen, 3′-methyl-4-dimethylaminoazobenzene, is relatively considerable. On the other hand, using the feebly carcinogenic 4′-methyl-4-dimethylaminoazobenzene and the non-carcinogenic 2-methyl compound, no more than a possible trace of bound dye in the ghost cell substance was found. A repeat experiment did, however, show some, though less, binding with both compounds. Under these experimental conditions the binding is not therefore specific to a carcinogen. Feeding of 4-dimethylaminoazobenzene for a long period resulted in the production of enlarged and grossly abnormal livers showing early neoplastic changes. No bound dye was detected in the ghost cell preparation from these livers.

The yield of ghost cell substance, expressed as percentage of fresh liver, from all the rats injected with azo-dye was strikingly reduced. Only in the case of the repeat experiment with 4′-methyl-4-dimethylaminoazobenzene and the 2-methyl compound could this reduced yield be ascribed, and even then only partially, to decreased cellularity of the liver as indicated by the concentration of liver deoxyribonucleic acid. In the other experiments, and notably

Table 1. Firm Binding to the 'Ghost Cell' Substances in Livers of Rats Treated with Azo-dye

Exp. No.	Treatment	No. of rats	Time killed	Bound dye: Absorbance/50 mgm. protein	
				Total liver	Ghost cell substance
1	3′-Me-DAB	8	41 hr.	0·183	0·129
2	3′-Me-DAB	6	48 ,,	0·176	0·085
3	4′-Me-DAB	2	43 ,,	0·024	Trace ?
4	2-Me-DAB	1	48 ,,	0·129	Trace ?
5	4′-Me-DAB	7	48 ,,	0·063	0·028
	2-Me-DAB	4	48 ,,	0·122	0·067
6	DAB	3	15 months	—	0·003

Exps. 1–5: Intraperitoneal injection, 20 mgm./100 gm. body weight in arachis oil. Similar numbers of control rats injected with arachis oil only.

Exp. 6: Fed 0·06 per cent 4-dimethylaminoazobenzene in stock diet. Similar numbers of controls fed stock diet only.

Absorbance is $\log_{10} I_0/I$, 1 cm. at 520μ for 4-dimethylaminoazobenzene (DAB), 525μ for 2-methyl-4-dimethylaminoazobenzene (2-Me-DAB) and the 3′-methyl compound (3′-Me-DAB), 530μ for 4′-methyl-4-dimethylaminoazobenzene (4′-Me-DAB).

with the 3′-methyl compound, a reduced concentration of liver deoxyribonucleic acid did not accompany the reduced yield of ghost cell substance.

It may perhaps be significant that there was a reduction in ghost cell substance in the 'neoplastic' livers resulting from long-term feeding with 4-dimethylaminoazobenzene. The livers from dye-fed rats were about twice the size of the controls, which might reflect a very low content of ghost cell substance in the neoplastic cell itself. Incidentally, the higher yield of ghost cell substance in this experiment was due to a less rigorous application of the extraction procedure in a first trial of the method. The reduced yield of ghost cell substance from the livers of rats injected with 3′-methyl-4-dimethylaminoazobenzene was accompanied by a reduced concentration of ribonucleic acid in the liver, which was most apparent when the animals were killed 48 hr. after injection of the dye. Since much of the cellular ribonucleic acid is associated with the endoplasmic reticulum to form the ergastoplasm, it would appear that dye injection has produced a loss in both components. This acute loss does not appear to be related to carcinogenic potency.

During the course of the present work, it was reported[4] that following feeding to rats, 3′-methyl-4-dimethylaminoazobenzene was firmly bound to the liver ergastoplasm and exclusively to the 'insoluble residuum' fraction thereof. It seems to us probable that this fraction and our ghost cell preparation represent the same cellular substance, that is, the structural lipoprotein of the endoplasmic reticulum, and that we have confirmed in another, possibly more direct, way that binding of carcinogenic azo-dye to this substance occurs. It is also stated in the report[4] already mentioned that single massive doses of dye may decrease the activity of (liver) glucose-6-phosphatase, an enzyme located in the 'insoluble residuum' fraction. If this is due to loss of endoplasmic reticulum, it would be in line with our finding of a diminished yield of ghost cell substance.

Table 2. Effect of Azo-dyes on the Yield of 'Ghost Cell' Substance and Concentration of Nucleic Acids in Rat Liver

Treatment	Dry liver/wet liver (per cent)	Yield ghost cell substance (per cent)	Composition of ghost cell substance			Liver nucleic acid conc.: change in dye group vs. control	
			Phosphatide (per cent)	N (per cent)	P (per cent)	RNA (per cent)	DNA (per cent)
3′-Me-DAB	23·2	0·30	—	—	—	−17·2	+14·1
Control	23·0	0·83	13·7	10·5	0·90		
3′-Me-DAB	25·0	0·25	7·9*	8·8	0·89	−35·5†	+3·0†
Control	25·7	0·93	10·2*	10·0	0·87		
4′-Me-DAB	24·2	0·52	—	11·9	0·91	+12·4	+7·3
Control	24·5	—	—	—	—		
2-Me-DAB	22·0	0·45	—	—	—	−18·5	+17·2
Control	25·8	1·31	10·3	9·2	0·95		
4′-Me-DAB	24·0	0·65	9·8	10·6	0·83	−6·7	−27·9
2-Me-DAB	22·6	0·26	11·1	9·7	0·83	−10·1	−20·4
Control	23·6	1·14	8·9	8·4	0·81		
DAB	—	1·58	13·1	10·5	9·94		
Control	—	2·45	10·0	9·3	0·83		

* Alcohol extractable only.

† Corresponding figures for additional groups treated similarly were −30·1 and +11·0 respectively.

Our results indicate that binding occurs in the region where tissue-specific antigen(s) is located. A liver microsomal sub-fraction containing a large proportion of the microsomal protein and phospholipid and thought to be a lipoprotein forming an integral part of the endoplasmic reticulum was shown by Vogt[8] to contain all, or nearly all, the tissue-specific antigen. Our preliminary results indicate that the major portion of firmly bound dye in the microsomes after injection of 3′-methyl-4-dimethylaminoazobenzene is in this fraction which contains nearly all the microsomal phosphatide (now completely confirmed in each of four experiments). This, together with the earlier findings, make it highly probable that tissue-specific antigen is involved in the binding process. The question of specificity must remain open until we know whether there is a big quantitative difference between carcinogens and non-carcinogens either in immediate binding, or in the time it persists, or both. However, present results, together with preliminary work on a transplantable hepatoma, indicate that the microsomal lipoprotein complex responsible for binding may be lost during carcinogenesis, since no binding occurred in the late stages of the process nor in the hepatoma. Failure of carcinogenic dye to bind with the soluble protein fraction from chemically induced hepatoma[8], whereas it shows firm binding in normal liver, may be secondary to the deficiency of tissue-specific antigen postulated by us. According to Hultin[3], primary binding is in the microsome fraction, followed by the release of metabolic products into the cytoplasmic fluid, which are then trapped by soluble protein components. If this is so, then in the absence of microsomal binding so-called 'specific' binding to the soluble protein fraction would not occur.

We have for some years[2], on the basis of much varied work, maintained that the cancer cell lacks in some degree cytoplasmic tissue-specific antigen which results in a loss of immunological identity as a tissue. This results in failure of certain (for example, 'scavenger') cells to recognize displaced cells, and in cancer cells lacking the receptors to recognize humoral (for example, hormonal) controls. The cancer cells can therefore no longer be fully integrated into an organized tissue or into the whole organism. The mechanism of loss of tissue-specific antigen doubtless varies greatly, but in chemical carcinogenesis immune attack on haptene-bound tissue-specific antigen may be important[2]. Even if this is not so, the basis of the immunological theory would not be affected.

We have previously suggested[10] that the tissue-specific antigens are membranous lipoprotein complexes and indicated how their loss could account for known alterations in the surface properties of the cancer cell. The concept that cellular affinities (that is, the forces binding like cells to like) are mediated

through antigens on cell surfaces is supported by the work of Spiegel[11], who prevented re-aggregation of marine sponge and amphibian embryonic cells by treatment with specific surface-active antibodies. The fact that exposure to a carcinogen has a somewhat similar effect on marine sponge cells[12] favours the idea that surface antigens are involved in carcinogenesis. We are now getting a clearer picture of the site and nature of such antigens and indications of how carcinogenic chemicals and agents of many different kinds interact with them and cause their loss[13].

There is evidence that the ergastoplasm is concerned with the synthesis of proteins peculiar to that cell, and ideas suggesting that differentiation and structure are controlled by self-replicating particles located in the ergastoplasm and linked to nuclear genes by it, have some experimental support[14]. The present findings provide evidence that carcinogens are able to bind firmly to the cell framework. It remains to be seen whether there is in this respect a quantitative, or even qualitative, difference between carcinogens and related non-carcinogens If there is, and absence of such binding in cancer tissue is confirmed, the idea that deficiency of tissue-specific antigens and neoplasia are synonymous terms will receive further support.

[1] Green, H. N., *Brit. Med. J.*, ii, 1374 (1954).
[2] Green, H. N., *Ann. N.Y. Acad. Sci.*, **68**, 268 (1957); "Cancer", **3**, edit. Raven (Butterworth, London, 1957); Ciba Foundation Symposium on Carcinogenesis, 131 (1959).
[3] Hultin, T., *Exp. Cell. Res.*, **13**, 47 (1957).
[4] Fiala, S., and Fiala, A. E., *Brit. J. Cancer*, **13**, 236 (1959).
[5] Bruemmer, N. C., and Thomas, L. E., *Exp. Cell Research*, **13**, 103 (1957).
[6] Smith, J. T., Funches, A., Barak, A. P., and Thomas, L. E., *Exp. Cell Research*, **13**, 96 (1957).
[7] Miller, E. C., and Miller, J. A., *Cancer Res.*, **7**, 468 (1947).
[8] Vogt, P., *Nature*, **182**, 1807 (1958).
[9] Gelboin, H. V., Miller, J. A., and Miller, E. C., *Cancer Res.*, **18**, 608 (1958).
[10] Green, H. N., and Wilson, R., *Nature*, **182**, 1054 (1958).
[11] Spiegel, M., *Ann. N.Y. Acad. Med.*, **40**, 337 (1954–55).
[12] Kennedy, G. K., 36th Ann. Report, Brit. Emp. Cancer Comp., p. 386 (1958).
[13] Green, H. N., *Acta Unio contra Cancrum* (in the press).
[14] Brachet, J., "Biochemical Cytology", 36–47 (Acad. Press Inc., New York, 1957).

THE BIOCHEMISTRY OF THE EYE*

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BIOCHEMICAL processes in the tissues of the eye are as diverse as in any other organ, and the functions of transmission of, and sensitivity to, light add special complexities. Size and shape must be controlled rigidly in order that the eye can act as an optical instrument, the tissues must be transparent, which means that they must be avascular, and the light-sensitive pigments in the retinal cells must be backed by the light-absorbing melanin of the choroid. Cornea, lens and vitreous body are light transmitters and focusers; the retina is the light-receiving and light-sensitive part of the eye.

Cornea and Lens

Recent experiments have shown that the clarity of the cornea depends upon its metabolism, which regulates the ionic balance. If respiration of the epithelium of the cornea is inhibited by fitting a type of contact lens, the epithelial cells swell and water accumulates in the fibrous stroma, thus making the cornea optically imperfect. Presence of oxygen beneath the contact lens prevents this swelling. Similar swelling occurs if the cornea is cooled or stored anaerobically or in the absence of glucose, but can be reversed when the tissue is re-warmed in presence of oxygen and glucose. A degree of swelling that might be unnoticeable in another tissue can distort the optical perfection of the cornea.

The cornea consists of three parts—epithelium, collagenous stroma and endothelium. The stroma is, in most animals, the largest part, but the epithelium has the most active metabolism. The cornea is avascular and oxygen is supplied by diffusion from the air, and, together with other nutrients, from the aqueous humour and surrounding tissues. The metabolism of glucose by the corneal epithelium appears to be similar to that of other tissues. The Qo_2 is 6–8, which is comparable to liver; cytochrome oxidase can be demonstrated histologically and cyanide inhibits respiration almost completely. Anaerobic glycolysis and some aerobic glycolysis occur. The enzymes of the citric acid cycle are present and glucose is also directly oxidized by the pentose phosphate pathway.

Glucose breakdown is the main source of energy in both cornea and lens. The relative importance of different paths of glucose breakdown has been studied in both tissues by Kinoshita and Wachtl using glucose labelled with carbon-14 on C_1 or C_6 or uniformly labelled on all carbons. In these avascular tissues anaerobic glycolysis is, as one might expect, the main path. The corneal epithelium glycolyses 65 per cent of all glucose metabolized and oxidizes 35 per cent, most of it by direct oxidation through the pentose phosphate route. The lens, which is much less active metabolically than the cornea, glycolyses almost all glucose used. The citric acid cycle is almost inactive in lens and any oxidation of glucose takes place by direct oxidation of C_1 through the pentose phosphate path. This oxidation is accompanied by reduction of triphosphopyridine nucleotide, and systems which can re-oxidize this coenzyme have been sought in both cornea and lens. Kinoshita found that the corneal epithelium contains a lactic dehydrogenase that will use reduced triphosphopyridine nucleotide, reducing pyruvic to lactic acid, and that addition of pyruvate anaerobically to cornea stimulates direct oxidation of glucose. Such a system may also be active in lens. Both tissues also contain a high concentration of reduced glutathione and glutathione reductase, which uses reduced triphosphopyridine nucleotide as coenzyme; but no system oxidizing glutathione has yet been found.

* Substance of two lectures given in University College, London, on February 29 and March 7, 1960.

The corneal stroma consists of fine uniformly arranged collagen fibres embedded in a matrix of mucopolysaccharides and mucoproteins. As these substances occur in the cornea unmixed with other fibrous proteins or with blood vessels it seems a suitable tissue in which to study collagen and mucopolysaccharide formation. Experiments have shown that repair of collagen in the cornea requires ascorbic acid. Three mucopolysaccharides are present—keratosulphate, a polymer of N-acetylglucosamine, galactose and sulphate, chondroitin sulphuric acid, a polymer of N-acetylgalactosamine, glucuronic acid and sulphate and the non-sulphated polysaccharide chondroitin. Cornea, both *in vivo* and *in vitro*, will incorporate sulphur-35 labelled inorganic sulphate into mucopolysaccharides. The uptake is much reduced in scorbutic guinea pigs.

The lens cells are derived from embryonic ectoderm. A small clump of cells grows into the optic cup, rounds itself off and becomes detached from the outer surface. By the time the human embryo is 2 cm. long this little clump has surrounded itself with a capsule which is impermeable to cells and to proteins, and the lens exists for the rest of life cut off from blood vessels and nerves, suspended radially in the eye by the suspensory ligament and bathed by the aqueous and vitreous humours. The adult lens has a dry weight of 35 per cent and nearly all of this is protein. The protein of the lens is not species specific. Common antigens are present in the lenses of all mammals examined, and man and animals can form antibodies to their own lens proteins. Escape of protein from a cataractous lens occasionally leads to development of sensitivity to lens protein and this can cause an intraocular allergic reaction. Until recently the lens was thought to contain only three major proteins, but with advance in electrophoretic techniques thirteen protein fractions have been separated and five fractions crystallized.

Senile cataract occurs throughout the world in man and animals but the biochemical changes are not known. A form of experimental cataract in which some of the biochemical changes are known is that produced in young rats by feeding high concentrations of galactose or xylose or by inducing diabetes with alloxan. R. van Heyningen has found that enzymes are present in the normal lens which will convert glucose to fructose by way of sorbitol. Sorbitol, present in traces in normal lens, accumulates in the lens of the diabetic rat, and the corresponding polyols, dulcitol and xylitol, accumulate in the lens of the rat fed galactose or xylose. The concentration of dulcitol may reach 2 gm./100 gm. lens after 1–2 days on a diet of 35 per cent galactose before any sign of cataract has appeared.

Cataract due to excess galactose also occurs in man. Some babies cannot metabolize galactose, do not thrive on milk and, among other signs of illness, may develop cataracts. Kalckar has found that galactosæmia is an inborn error of metabolism and that these babies do not have the enzyme galactose-1-phosphate uridyl transferase in their tissues, and their red blood corpuscles contain galactose-1-phosphate. Schwarz and Golberg found traces of galactose-1-phosphate in the fully cataractous lenses of galactose fed rats, and Lerner found that lenses from a galactosæmic infant had a low galactose-1-phosphate uridyl transferase activity. Whether block of galactose breakdown at the dulcitol-level with early high accumulation of dulcitol or block of the further metabolism of galactose-1-phosphate causes lens opacity to develop is still not clear.

Such cataracts are very rare in man, but senile cataract is still listed as the largest single cause of blindness in the world as a whole, in spite of the fact that the blindness can be relieved by surgical removal of the cataractous lens.

The Vitreous Body

The vitreous body fills the space between the lens and retina and can be considered as an extremely tenuous connective tissue. Although it is 99 per cent water it is a surprisingly stable jelly-like structure which does not dissolve when suspended in saline. A fibrous insoluble residue was described by Mörner in 1894, who suggested that it might be collagen, and this was confirmed using chromatographic and X-ray analysis. Electronmicrograms show very fine uniform fibres which occasionally show a cross-striation of 640 Å., in the posterior part of the vitreous body, while coarser fibres are present near the lens where the vitreous body is very firmly attached to the retina and outer coats of the eyeball. When the fibrous part is separated by centrifugation or filtration a clear viscous fluid remains, the vitreous humor, from which hyaluronic acid was first isolated by Meyer and Palmer in 1934. The stability of the vitreous body depends on the network of collagen fibres permeated by polymerized hyaluronic acid. The concentration of hyaluronic acid in the vitreous of cattle is about 400 mgm./l., and the concentration of fibrous protein 150 mgm./l., yet together, these large molecules, even in these low concentrations, form a surprisingly stable structure.

The Retina

The retina, like the brain from which it is derived embryologically, has a very active metabolism. The Qo_2 is about 10, and glycolysis proceeds both aerobically and anaerobically. Oxidation of glucose takes place by the pentose phosphate path and by the citric acid cycle, judged by formation of carbon dioxide from glucose labelled with carbon-14 on C_1 or C_6. But in studies of yields of radioactive lactic acid, carbon dioxide or other intermediates the fact that retina will actively incorporate carbon dioxide into malate must be remembered. Addition of pyruvate to retinal tissue stimulates direct oxidation of glucose presumably through stimulation of reactions which re-oxidize reduced triphosphopyridine nucleotide, thus supplying triphosphopyridine nucleotide for glucose oxidation. Such reactions could be the formation of malate from pyruvate plus carbon dioxide catalysed by the malic enzyme or reduction of pyruvate to lactate catalysed by a lactic dehydrogenase.

Attempts have recently been made to correlate the high rate of retinal glycolysis with maintenance of light sensitivity, and to determine metabolic changes when the retina is illuminated. Injection of iodoacetate in many species of animals causes an almost immediate fall in the electrical response of the retina to light and this is followed by a progressive fall in glycolysis with degeneration of those cells in the retina which contain light-sensitive pigments. But further work has shown that iodoacetate inhibits other processes in the retina besides glycolysis. No change has been found in respiration, in adenosinetriphosphate, deoxyribonucleic acid or ribonucleic acid when the retina is illuminated.

The main biochemical study of the retina has always been study of the light-sensitive pigments themselves. This brief review can discuss only one class, namely, those which are contained in the rod cells of vertebrate retinas and are thought to be the basis of vision in dim lights where colours are not appreciated. These pigments, the rhodopsins and porphyropsins, are lipoproteins with carotenoid chromophores, the carotenoid being retinene A_1, the aldehyde of vitamin A_1, or retinene A_2, the aldehyde of vitamin A_2. It is interesting that the first connexion between vitamin A and visual pigments came from study of human and experimental night blindness and that later biochemical work showed the vitamin to be a part of the pigment molecule. Rhodopsin can be isolated from the rod cells by extraction with digitonin with which it forms a soluble complex. It is a red pigment which fades to a yellow when exposed to light. This red pigment was first seen in the frog retina by Boll in 1876, who noticed that it faded in light but was re-formed if the live animal or separated eye was left in the dark.

The breakdown of rhodopsin by light and its reformation in darkness has been studied by examination of changes in the absorption spectrum. Mammalian rhodopsin has an absorption maximum at 500 mμ, which is also the wave-length of light to which the mammalian eye is most sensitive. Change in absorption at 500 mμ measures change in rhodopsin. The primary action of light can be studied by determining change at very low temperatures, − 70° C., where thermal reactions are stopped. Such experiments show that light does not, strictly speaking, bleach rhodopsin but only changes the point of maximal absorption very slightly to the blue. Subsequent reactions which occur in light or in the dark cause more profound changes with release of retinene. Recent work of Wald and Hubbard has suggested that the retinene present in rhodopsin is a particular 11-*cis* stereoisomer and that the action of light is to change this isomer to the all-*trans* form while leaving it still attached to the protein. Vitamin A and retinene in other parts of the body are the all-*trans* isomers, but the pigment epithelium in mammalian eyes and the eyes of some marine crustaceans contain 11-*cis* retinene. The mechanism of formation of this isomer in the eye is not clear. Wald found it less effective than the all-*trans* isomer in promoting regeneration of rhodopsin in the retina of the vitamin A-deficient rat.

Examination of the density of rhodopsin in the living eye has shown that visual sensitivity is not related directly to rhodopsin concentration. Bleaching half a per cent of the total rhodopsin reduces sensitivity three-thousand-fold. Probably neural factors as well as pigment concentration are involved.

Apart from the light-sensitive pigments of the visual cells there are other pigments in the eye that modify the light stimulus received. For example, there is a yellow pigment, probably the carotenoid xanthophyll, at the macula, where vision is most acute in the human eye. This absorbs about 60 per cent of all incident blue light. The retinal pigment epithelium or choroid of many fish, amphibians and at least one reptile contains fluorescent pterins, and similar parts of the eyes of other species of fish and some mammals are rich in riboflavin. The most extreme example is the eye of the lemur, *Galego crassicaudatus*, in which there is a layer of crystals of riboflavin immediately behind the visual cells. This mirror will both reflect light and, through its fluorescence, will transform short-wave blue light to light of longer wave-length (520 mμ) to which rhodopsin is more sensitive.

Many animals have elaborated mirrors behind their retinas or on their irises. Carnivores have a layer of crystals of zinc cysteine ; and in the silver fox the zinc concentration of this part of the eye may reach 16 per cent of the dry weight. This is also the basis of the eye shine of dogs and cats. Fish and amphibians lay down crystals of guanin as a mirror in their eyes as in their scales (fish silver), while lemurs have crystals of riboflavin. The biochemical processes whereby these substances are concentrated to such an extent that they crystallize have yet to be investigated.

The study of both the specialized and the general biochemistry of eye tissues is only just beginning to expand. It is a fascinating study, and should lead to a clearer understanding of normal and abnormal processes in the eye as well as giving information which is interesting in connexion with the metabolism of other tissues.

OBITUARIES

Prof. H. W. B. Skinner, F.R.S.

Herbert Skinner, Lyon Jones professor of physics in the University of Liverpool, died suddenly in Geneva on January 20, at the age of fifty-nine, leaving a wife and daughter. His death was a great shock to a wide circle of friends and colleagues, for he was well known and liked far beyond his own University.

He was educated at Rugby and Trinity College, Cambridge, and worked under Rutherford at the Cavendish Laboratory until 1927, when he joined Tyndall at Bristol. He was elected to the Royal Society in 1942 in recognition of his work on the soft X-ray spectroscopy of metals.

Early in the War, Skinner worked on radar at the Telecommunications Research Establishment, which was a remarkably successful amalgamation of academic, industrial and Government scientists working in close liaison with the communications industry and the Royal Air Force. Skinner was one of the bright spirits of this enterprise and certainly contributed much to its success. He left in 1943 to join the atomic weapons work under the Department of Scientific and Industrial Research, and went to California as a senior member of the British group working with E. O. Lawrence and his colleagues on the electromagnetic separation of uranium isotopes.

Skinner returned to act as deputy to Sir John Cockcroft, who was still in Canada, in setting up the Atomic Energy Research Establishment at Harwell. He and a few colleagues began the creation of a great research laboratory from an empty R.A.F. station on the edge of the Berkshire Downs. Looking back now, it is remarkable that such impressive progress was made in those early years, when conditions were

so difficult. Skinner's contribution, first acting in Cockcroft's absence and later as one of his able lieutenants, was a most important one. His particular personal creation was the General Physics Division which, organizationally, could have no logical justification but was a reflexion of his own wide interests and was extremely productive. Under his personal supervision came the synchrocyclotron, cloud chamber and nuclear emulsion work, synchrotrons, linear accelerators and electronics instrumentation, X-ray and neutron diffraction, isotope separation and mass spectrometry, and, later, extra-mural work on plasma physics. All this work prospered and, with the growth of Harwell, some found homes in new divisions.

Four years later, when chief physicist at Harwell, Skinner was appointed to succeed Sir James Chadwick at Liverpool. There he had further opportunity to exploit his talent for construction, because Chadwick, having created a flourishing school of nuclear physics around one of the first cyclotrons in Europe, had undertaken the construction of a new research laboratory centred on a 156-in. synchrocyclotron. This large project was in an early and critical stage, but was brought to a highly successful conclusion with the help of Chadwick's former colleagues and new men brought in by Skinner. Unlike Chadwick, Skinner was not primarily a nuclear physicist, and he appointed J. M. Cassels as professor of experimental physics. Liverpool became the home of a powerful school of high-energy physics, and the research work more than kept pace with the general growth in the universities. The small cyclotron, together with a Cockcroft–Walton generator, enabled the traditional nuclear physics school at Liverpool to grow in size and output to such good effect that one of Skinner's last acts was to undertake the installation of a tandem electrostatic generator for further expansion in this field. This machine is still being manufactured, and it is tragic that Skinner will not see it installed. A new and impressive building, the Chadwick Physics Laboratory, for teaching and research was completed recently under his guidance.

Skinner was active in the work of the European Organization for Nuclear Research (CERN) and was the senior British consultant in the 600-MeV. synchrocyclotron project. He was chairman of the CERN Panel of the Department of Scientific and Industrial Research, concerned with British participation in CERN'S work, and served on the Nuclear Physics Sub-Committee. He remained keenly interested in atomic energy, and was a consultant to the Atomic Energy Authority and a member of the *Nimrod* Project Committee. The National Institute for Research in Nuclear Science has good reason to be grateful for his advice and help on numerous occasions, and he was an active and provocative member of several committees of the Institute. He brought his laboratory into the national Liquid Hydrogen Bubble Chamber project sponsored by several British universities.

Herbert Skinner was devoted to physics and to physicists, and was generous with his time and energies. He hated red tape and sometimes alarmed administrators. Unconventional, persistent in argument and often disconcerting in management, he was also sincere, gentle and kind to an extent not always understood by his critics. He and his wife Erna were generous in hospitality, and their home was often crowded with guests. The affairs of physicists are duller without him.

T. G. Pickavance

Prof. Marcel Schein

On February 20, at the height of his scientific career, Marcel Schein died in Chicago, Illinois. With him disappeared a most brilliant, able and imaginative scientist whose work is destined to leave an enduring mark in the physics of our century.

Marcel Schein was born in Tresten, Czechoslovakia, on June 9, 1902. After studying in Prague, Vienna, Würtzburg and Zurich, he received a doctorate in physics at the University of Zurich in 1927. His first academic appointment was that of assistant in physics in that University. Between 1929 and 1930 he spent a year in Chicago on a Rockefeller Fellowship. He then returned to Zurich as a *docent*, a position which he held until 1935, when he was appointed professor of physics at the University of Odessa. In 1938, Marcel Schein went to settle permanently in the United States, at the invitation of Prof. A. H. Compton. He joined the University of Chicago where he remained until his death, holding various positions until, in 1946, he was appointed professor in the Department of Physics and in the Institute of Nuclear Studies. In 1927, while in Zurich, he married the former Hilde Schoenbeck, who was working as a Ph.D. candidate in the Physics Department. From this marriage he had a son, Edgar, who is now on the faculty of the School of Industrial Management at the Massachusetts Institute of Technology.

In the early years of his scientific career, Marcel Schein made some valuable contributions to the physics of X-rays. He is best known, however, for his work in cosmic rays, which he began after moving to Chicago in 1938. At that time it was known that the primary radiation consisted, at least to a large extent, of positively charged particles. The possibility that these particles might be protons had been suggested ; however, there was no direct experimental evidence to support this assumption. Indeed, the prevailing view was still that primary cosmic rays were for the most part positive and negative electrons. During 1938–41, Marcel Schein conducted a series of balloon experiments in which he studied the physical properties of cosmic-ray particles in the highest layers of the atmosphere and was able to show that these particles did not behave in at all the way that high-energy electrons were known to behave. He concluded that electrons did not appear in any appreciable amount among primary cosmic rays, and that the incoming radiation consisted most likely of protons[1].

These experiments remain one of the milestones of cosmic-ray research. However, they were only the beginning of Marcel Schein's work in this field of science. Soon his interest became focused on the most energetic particles present in the primary cosmic radiation and on the remarkable and unbelievably intricate processes that occur when these particles strike atomic nuclei. Marcel Schein was a pioneer in the study of nuclear interactions at energies far exceeding those provided by man-made machines. At the time of his death, he had just returned from an expedition to the Caribbean where, in an effort to extend further and improve the experimental data on high-energy interactions, he had flown the largest stack of nuclear emulsions ever used in any experiment. This stack, which is now being processed, will undoubtedly contain invaluable data the analysis and interpretation of which will keep many physicists busy for a number of years.

Marcel Schein was keenly interested in international scientific collaboration. He travelled extensively in foreign countries, providing great stimulation through his lectures and his informal contacts with scientific workers. He participated most actively in practically all major international conferences dealing with subjects within his field of interest. His last experiment, which I mentioned before, was a truly international venture, with a large number of laboratories, both in the United States and abroad, participating in the analysis of the experimental results.

Marcel Schein was, in many ways, a remarkable man. For him science was a passionate search for the new and the unknown, involving all his scientific skills, as well as his deepest emotions. His sharp mind, his keen intuition, his irresistible enthusiasm and his intense personality will be long remembered by his many friends, pupils, and professional associates in all parts of the world. BRUNO ROSSI

[1] Schein, M., Jesse, W. P., and Wollan, E. O., *Phys. Rev.*, **59**, 615 (1941).

Prof. G. P. Mazumdar

PROF. G. P. MAZUMDAR died suddenly on November 21. He was born on February 18, 1894, at Gopalnagar in the District of Pabna, North Bengal (now in East Pakistan), the third son of Kali Charan Mazumdar, a wealthy landlord and Zeminder of the district. After obtaining his B.Sc. at the University of Calcutta in 1913, he joined the teaching staff of the Botany Department, Presidency College, Calcutta. He later obtained his B.A. (1916) and M.Sc. in botany (1915), standing first in order of merit, and in 1917 the B.L. degree from the same University. He was eventually appointed a professor of botany in the Bengal Educational Service in the Presidency College, Calcutta, and from 1928 became one of the postgraduate lecturers in botany at the University of Calcutta. In 1938 he went on study leave to England to work on plant anatomy in the University of Leeds under the late Prof. J. H. Priestley and obtained the degree of Ph.D. in 1940. He retired from the Government service in 1949; but his services were soon requisitioned by the Government of West Bengal for the organization of honours teaching in botany in the Government College, Darjeeling. In December 1951 he was appointed professor of botany and head of the Biology Department, University of Dacca, in East Pakistan, and retired in October 1954. While in Pakistan, he acted as the chairman of the Commission of Courses of the Karachi University and presided over the Section of Biology at the annual session of the Pakistan Association for the Advancement of Science in January 1955. In 1945 he presided over the Section of Botany at the thirty-second Indian Science Congress. He also presided over the Section of Technical Sciences at the All-India Oriental Conference some few years back. He was elected a Fellow of the Indian Academy of Sciences, Bangalore, and National Institute of Sciences of India (1945). He was for a long time connected with the Indian Association for the Cultivation of Science, now at Jadavpur, Calcutta, both as a member and a treasurer. He was also member of other Indian learned societies.

Prof. Mazumdar published a large number of scientific papers in numerous journals throughout the world and several scientific Bengali primers, especially in botany. His writings for children on scientific subjects and botany in Bengali have evoked admiration. He also dealt with botany in ancient India in several books and papers. His "Vanaspati" earned for him the Griffith Memorial Prize of the University of Calcutta in 1925. His "Upavana Binoda" (dealing with horticulture in ancient India) was published in 1938. He published other works on the history of botany. The Asiatic Society awarded him the P. Bruhl Memorial Medal in 1955 for his outstanding researches in botany.

R. M. DATTA

NEWS and VIEWS

Botany at Cambridge: Prof. G. E. Briggs, F.R.S.

ON September 30, Prof. G. E. Briggs will retire from the professorship of botany in the University of Cambridge. Since he succeeded Prof. F. T. Brooks in 1948, he has maintained the notably wide range of teaching and research in the Botany School and has fostered expansion in several branches of the subject. The Department has flourished under his direction, and has benefited greatly from his example of easy friendliness. As a physiologist he has always believed in the value of a quantitative and critical approach both in teaching and research, and his lectures were planned not only to show this but also to indicate the weaknesses of many currently accepted generalizations. He is a brilliant teacher, and for those undergraduates and research students who could rise at least part-way towards his high standard, his lectures and supervision provided a never-to-be-forgotten education. His researches on enzyme kinetics, on growth and photosynthesis early brought him a world-wide reputation as a critical and original thinker. More recently he has entered a new field of research, the biophysics of the plant cell, and his success in this work has drawn to him a flourishing group of research students. In 1952 he became president of St. John's College, but he never allowed the duties of this post to curtail the time he gave to the Botany School.

Prof. H. Godwin, F.R.S.

DR. H. GODWIN, reader in Quaternary research at Cambridge, who has been appointed to succeed Prof. Briggs, is known to a large number of former Cambridge students as an able and enthusiastic teacher of botany at all levels. Outside Cambridge, he may be best known for his large book on the Quaternary history of the British vegetation. His first research was, in fact, in pure plant physiology with Dr. F. F. Blackman, but from early days the inspiration of A. G. Tansley led him to work at Wicken Fen and eventually ecology took up his whole time. Study of fluctuating water-level in the peat and of the effects of this and of cutting reeds on fen vegetation soon directed his attention to the history of this vegetation in the immediate past, and to actual changes in this locality rather than general seral development. Thus he naturally dug in the peat for visible plant remains, and was soon using the methods of pollen

analysis worked out not long before by von Post in Sweden. He gradually developed his own blend of the study of pollen, other plant remains and local ecology. He has now a considerable and well-equipped research school. Latterly their work on the more recent peats have been telling such consistent stories that his school have pushed their studies back into the Late Glacial and into the more extensive wilderness of the various Interglacials.

Organic Chemistry in the University of New South Wales : Prof. S. J. Angyal

THE newly created chair of organic chemistry in the University of New South Wales (formerly the New South Wales University of Technology) has been filled by the appointment of Dr. S. J. Angyal, formerly associate professor in the University. Prof. Angyal was educated in Hungary and migrated to Australia at the beginning of the War. As lecturer in the University of Sydney, he carried out research on the Sommelet reaction. In 1952, as Nuffield Dominion Travelling Fellow, he spent a year in Sir Alexander Todd's laboratory in Cambridge. He was visiting professor in the University of California in Berkeley in 1957. In the following year he was awarded the H. G. Smith Memorial Medal by the Royal Australian Chemical Institute. Prof. Angyal takes great interest in stereochemistry, and has made important contributions to the chemistry of the inositols. At present he is engaged in work on the application of conformational analysis to carbohydrate chemistry.

Radio Research in Britain : Dr. J. A. Saxton

DR. J. A. SAXTON has been appointed deputy director of the Radio Research Station, Slough (Department of Scientific and Industrial Research), in the grade of deputy chief scientific officer. This appointment is opportune, as he will serve for a period of six months under the present director, Dr. R. L. Smith-Rose, before the latter is succeeded by Mr. J. A. Ratcliffe on October 1 (see *Nature*, February 6, p. 353). Dr. Saxton joined the scientific staff of the Radio Division of the National Physical Laboratory in 1938, and has been responsible for carrying out a considerable programme of research in the propagation of very short radio waves over the ground and through the troposphere. He has studied the electrical properties of gases, water vapour, and water, at very high radio-frequencies, and applied the results to an understanding of the refraction of radio waves transmitted through the lower atmosphere. During 1945, and again in 1950, he was seconded to the United Kingdom Scientific Mission in Washington for liaison duties in radio physics and engineering ; and he has made extensive visits in the United States and Canada in connexion with research in radio wave propagation and allied problems. He has been an official delegate of the United Kingdom at meetings of the International Scientific Radio Union (U.R.S.I.) and the International Radio Consultative Committee (C.C.I.R.) in London, Geneva, The Hague, Boulder (Colorado) and Los Angeles. Dr. Saxton has attended senior staff courses at the Administrative Staff College, Henley, and at Peterhouse College, Cambridge. For the past five years, he has been a senior principal scientific officer, and head of a division of the Radio Research Station, and so is well qualified and experienced for the new post of deputy director of the Station.

Atoms for Peace Awards for 1959 and 1960

DRS. LEO SZILARD, professor of biophysics in the University of Chicago, and Eugene P. Wigner, Thomas B. Jones professor of mathematical physics, Princeton University, are to share the 1959 Atoms for Peace Award ; and Dr. Walter H. Zinn, formerly director of the Argonne National Laboratory, and now vice-president of Combustion Engineering, Inc., and Dr. Alvin M. Weinberg, director of the Oak Ridge National Laboratory, will share the 1960 Award. All have been active in the development of nuclear reactors. Each will receive a gold medallion and will share equally in the combined honorarium of 150,000 dollars. The Awards will be presented at a ceremony to be held at the National Academy of Sciences, Washington, D.C., on May 18. In making the announcement, Dr. James R. Killian, jun., chairman of the Trustees of Atoms for Peace Awards, said :

"The Trustees believe the development of the nuclear reactor is one of the great advances in man's capability for using atomic energy for peaceful purposes. It gives the world a new source of energy with which to meet the growing requirements of modern society for power to run its machines. As a source of radioisotopes, it is now providing science and industry with new possibilities in research and control, as well as with new products. The transformation of a highly complex, theoretical concept of 1939 to the multitude of reactors operating to-day in research and in commercial establishments is a major achievement of modern science and engineering. The men who are selected for recognition by this Award have been leaders in that transformation. Dr. Szilard and Dr. Wigner, both working in the area of nuclear physics, were early advocates of a concerted effort to study the possibilities of nuclear chain reactions. Their interest and concern resulted in the well-known letter of the late Albert Einstein to President Roosevelt which noted that "the element uranium may be turned into a new and important source of energy in the immediate future". These two men held an influential place in the work which resulted in the first nuclear chain reaction and in the subsequent applications of that significant development. Dr. Zinn and Dr. Weinberg participated in the early work and have continued to make important contributions in the field of reactor design and in the administration of research and development efforts in this area."

The Atoms for Peace Award was established as a memorial to Henry Ford and his son, Edsel, in response to President Eisenhower's 1955 appeal at Geneva for international efforts to develop nuclear energy for peaceful purposes. It is granted "solely on the basis of the merit of contribution, wherever found in the world and without regard for nationality or politics".

Ballistic Missile Early Warning Station at Fylingdales Moor

THE debate in the House of Commons on March 25 on Mr. T. Driberg's motion that to establish a ballistic missile early warning station on Fylingdales Moor is contrary to the spirit of the National Parks and Access to the Countryside Act, 1949, is chiefly of interest for the reply of the Under-Secretary of State for Air, Mr. W. J. Taylor. Mr. Driberg argued that unless the National Parks Commission was consulted in advance, consultation meant nothing, and he also alleged that some scientific opinion held that the station could not be equipped with a system of radar

detection capable of identifying ballistic missiles with certainty. Mr. Taylor repeated the assurance given by the Secretary of State for Air on March 3 that no alternative area was available in Britain and said there was to be very full discussion of the detailed proposals between representatives of the Air Ministry, the National Parks Commission and the Council for the Preservation of Rural England, and while the siting of the radars themselves could not be modified, he hoped that by careful choice of design and attention to landscaping details, the appearance of the completed station should be such that the disturbance to the natural landscape would be generally acceptable. On the certainty of detection, Mr. Taylor said that the opinion quoted was not sustained by the advice of the distinguished scientists who had advised the Government on the project and that in the view of those scientists the system would work. He was informed that there was no difficulty in distinguishing between ballistic missiles and artificial satellites and that the possibility of confusion between a missile trajectory and an ionization trail was so remote that for practical purposes it could be ignored. The Government was satisfied that this system of radar detection was capable of identifying ballistic missiles with very great precision and, moreover, the station was complementary to other methods of obtaining information about hostile intentions and provided the last stage of tactical warning.

Development of Ballistic Missiles

On April 13 the Minister of Defence, Mr. H. Watkinson, in the House of Commons, and the First Lord of the Admiralty, Lord Carrington, in the House of Lords, announced that the Government has been considering the future of the project for developing the long-range ballistic missile *Blue Streak* and has been in touch with the Australian Government about it. The vulnerability of missiles launched from static sites, and the practicability of launching missiles of considerable range from mobile platforms have now been established, and in the light of military advice, the Government has concluded, and the Australian Government has fully accepted, that Britain should not continue to develop, as a military weapon, a missile that could be launched only from a fixed site. The Government is now considering whether the *Blue Streak* programme can be adapted for the development of a launcher for space satellites, and a further statement will be made as soon as possible. There are many other projects for which the range at Woomera will be needed, and a substantial programme of work for that range for some years to come at least is expected.

In reply to further questions Mr. Watkinson said that the cost of the project to date has been about £65 million and that of completing it would be between £500 and £600 million. None of the expenditure would be wasted if it was decided to go ahead with *Blue Streak* as a launcher for a space satellite. The Americans are investing an immense sum of money in space research, both for its military and its scientific and industrial value, and at present Britain could only get into space by the American offer to launch British satellites. *Blue Streak*, in conjunction with *Black Knight*, which has a record of firings practically 100 per cent perfect, is a possible space launching-vehicle, and this combination may give us experience of the utmost military and scientific value.

New Nature Reserve in Scotland

Strathy Bog, an area of 120 acres in the County of Sutherland, has been declared a nature reserve by the Nature Conservancy; this brings the total number of national nature reserves in Scotland to 24 with a total area of 101,809 acres. The new reserve is the least-disturbed part of a vast area of bogland which covers the flat or gently undulating moorlands of this district. The ground is extremely wet, with standing water in the pools throughout the year, and *Sphagnum* moss is prominent in the vegetation, giving a generally spongy surface. In a rich assemblage of bog-plants there are cotton-grasses, sundews, bog asphodel, dwarf birch and bearberry. The area shows certain unusual structural features. Part of the Bog is covered with elongated pools lying parallel to each other, giving the impression that the surface has torn repeatedly under tension caused by the rather fluid mass of underlying peat tending gradually to slide down-hill. Such bogs may thus eventually break up and dry out even when unaffected by human disturbance.

The Nature Conservancy also announces additions to the nature reserve at Rannoch Moor. The original area was 3,485 acres in the north-east corner of the Moor of Rannoch. The new area comprises 200 acres covering a stretch of Loch Laidon and a small area of 19 acres at the southern end of the existing reserve.

International Communications and Scientific Journals

With the rapid growth of new journals the problem of presenting these journals in a form which will make their contents available to readers in other countries becomes more and more acute. To help English-speaking peoples, an increasing number of editors in countries oversea are preparing copies of their journals translated into English. Although this practice is highly commendable from the point of view of English-speaking readers, the journals often fail to make the impact they might because of the inadequacies of the translation. It is extremely difficult to translate the highly technical language of one country into that of another, and, unless special precautions are taken, a translation may produce effects which are not quite what the original research worker had intended. Recent English versions of journals which emanated from France, Czechoslovakia and Japan all indicate that the translations have been carried out by natives of those countries who 'know English well'. Before publication they would have been well advised to submit their publications to English-speaking men of science who also have a good working knowledge of the foreign language concerned. In this way the circulation value of their journals would be considerably enhanced, and many ludicrous statements would be eliminated. Another solution to what is admittedly a most difficult problem might be greater use of an auxiliary language like Interlingua or Esperanto. A world-wide conference to discuss the matter could be of benefit to men of science everywhere.

Abstracting Scientific Information

The Review of the International Council of Scientific Unions for July 1959 contains an article by Prof. E. A. Boutry, secretary of the International Council of Scientific Unions Abstracting Board, describing the work of the Board, its story, ideas,

methods and aims, which merits wide reading by scientific workers. Prof. Boutry points out that owing to the rapid increase of the bulk of published scientific information, the probability that printing a paper in a periodical will suffice to ensure that the scientific information it contains will reach the appropriate scientific worker has steadily decreased in recent decades and is likely to continue doing so, nor have editors of abstracting periodicals any certain means of procuring all the literature they should review or learning of its existence. Needless repetition and duplication hamper the work of abstracting periodicals and no mechanical or automatic methods can improve the quality of the literature published or reviewed, or reduce useless duplication. Looking to the future, Prof. Boutry makes various proposals. Authors, he suggests, should voluntarily accept and comply with certain rules in writing their manuscripts. Particularly he advocates submission with the paper of an author's abstracts in two versions, one of which should be written in English or French, composed in accordance with the "Guide for the Preparation and Publication of Synopses" (Unesco). He also deprecates publication of incomplete and incorrect "Letters to the Editor". Editors are asked to agree voluntarily to see that these practices are followed and to waive copyright in respect of such abstracts for members of the Board, while national committees of science and national scientific societies are asked to help in tracking and listing serial or non-periodical publications in their countries and to discourage excessive printing of non-periodical publications.

Carnegie Trust for the Universities of Scotland

THE fifty-eighth annual report of the Carnegie Trust for the Universities of Scotland covers the year ended September 30, 1959, in which investment policy has absorbed an exceptional amount of the time of the Executive Committee (pp. iii+73. Edinburgh: Carnegie Trust for the Universities of Scotland, 1960). The new investment policy has substantially improved the income of the Trust, which reached a record high level of £176,564 in consequence. The largest single payment was £33,000 to the University of Glasgow towards the cost of the new arts building, while Edinburgh received £20,000 towards the cost of its new Staff Club. During the year there were on the books two Fellows, three senior scholars and 68 scholars of whom 30 were new appointments, and annual expenditure on the research scheme reached a record level of nearly £27,000. Counting mathematics and geography as arts subjects, forty of the awards were in arts and thirty-three in science; and 22 of the latter were to chemists, including biochemists. Under the new regulations, which discouraged applications from students benefiting from the improvement of local authority bursaries in 1957, while the number of beneficiaries fell from 1,430 in 1957–58 to 568 in 1958–59, the average payment per beneficiary rose from less than £15 to £30 and the total expenditure on fees only fell from £21,500 to just over £17,000. Appendixes to the report include the memorandum of evidence submitted to the Anderson Committee on Grants to Students and the usual reports on the work of Fellows and Scholars and on research grants other than travel grants as well as a list of publications since September 30, 1958, by Fellows, Scholars and recipients of grants. It is noted that three out of the six scholars who have completed their third year of work in chemistry have undertaken post-graduate research in the United States or Canada, but that information suggests that at least half such British research graduates in chemistry eventually return. Assistance given to the Scottish Dictionaries Joint Council was consolidated into an annual grant of £5,000 until 1963 and assistance to Scottish learned societies towards the cost of publications has been restricted to the present level of £2,500 per annum.

Instrument Abstracts

A USEFUL journal for all who work with instruments is compiled by the British Scientific Instrument Research Association (No. 1, Vol. 1, January 15, 1960). It contains details of a wide range of publications in instrument technology with abstracts of many of them. Besides a general section there are sub-sections on astronomy, atomic physics, biology and medicine, chemistry, control, data handling, electricity, electronics, fluid mechanics, geophysics and meteorology, heat, light, magnetism and electromagnetism, materials and design, mechanics, navigation, sound, surveying, and telecommunications. The publications listed have been taken from many countries including France, Germany, Holland, United States and the U.S.S.R. *Instrument Abstracts* is published monthly by Taylor and Francis, Ltd., Red Lion Court, Fleet Street, London, E.C.4. The annual subscription is £4.

U.S.S.R. and the International Geophysical Year

A SERIES of publications concerning various activities in the U.S.S.R. during the International Geophysical Year has been issued. Included are: a classified bibliography of 616 items covering all branches of activities in the U.S.S.R. during the International Geophysical Year ("Bibliographical Index of Literature in Russian Language for 1958." Pp. 63. Moscow: Publ. Acad. Sci. U.S.S.R.); a report on thirteen fulfilled plans of observations, four reports and some articles dealing with geophysical researches carried out with rockets in the United States (Information Bulletin No. 7. Pp. 114. Moscow: Publ. Acad. Sci. U.S.S.R.); a collection of twelve articles dealing with geomagnetism and ionosphere investigations ("Magneto-Ionosphere Disturbances". Pp. 75. Moscow: Publ. Acad. Sci. U.S.S.R.). In each case the text is in Russian; however, there is also a contents in English.

Russian for Scientists

A VERY slender pamphlet by Prof. J. W. Perry, entitled "Scientific Russian Without Tears", has been issued recently. Unfortunately it is far too short to be of much value to a novice and far too elementary for an advanced student of the Russian language (pp. ii+26. (Reprinted from *The Chemical Bulletin.*) (Chicago: Chicago Section, American Chemical Society, Inc., 1959.) 1 dollar). Its only possible use is as an auxiliary to a normal dictionary in the case of a scientist trying to read Russian scientific literature, but in this case a dictionary of scientific terms would obviously be better. It is "Without Tears" for the simple reason that the majority of words included are international, for example, atom, vector, canal, parameter, etc., and only a knowledge of the Cyrillic alphabet is necessary to read them in Russian. A novice who is struggling with the intri-

cate cases and tenses of the Russian language and cannot ask for a glass of water is invited to read sentences like "rubber similar to isoprene and other unsaturated hydrocarbons takes up bromine". The examples given in this book are mainly from the field of chemistry and physics. Scientists should not content themselves with only picking up a scientific jargon. Unless they know the fundamentals of the language they may misread a scientific article rather than read it. Once they have mastered ordinary Russian, the only additional book they will need is a suitable dictionary.

Films and Film Strips in Teaching

The steady success of the educational films and film-strips produced by Gaumont–British Instructional Films, Ltd., now called Rank Precision Industries, Ltd., was due to clear recognition that they could never be more than aids to learning. The place of the teacher was accepted as paramount and all films and film-strips produced were deliberately designed to support the teacher rather than supplant him. As a result of this policy, Rank Precision Industries, Ltd., is now recognized as a valuable partner by the teaching profession in providing a wide range of both still and moving films. The latest film catalogue describes more than six hundred films in their separate categories with information about their content, running-time, suggested age-group, classification and hire- and purchase-prices. Besides films produced by Gaumont British Instructional, details are given of films produced by Encyclopædia Britannica Films, United World Films, and other organizations. In the film-strip catalogue more than three hundred strips are listed under eighteen subject categories; many of the films are in colour. Both catalogues are attractively produced and can be obtained from the Gaumont-British Film Library, Aintree Road, Perivale, Middlesex.

'Araldite' in Aircraft Research Models

For aerodynamic research, model aircraft of great accuracy and strength are required. A paper, published for the Royal Aircraft Establishment, Bedford, by the Ministry of Supply as Technical Note No. *A.D.W.*6, describes the making of models with shells consisting of 'Araldite' glasscloth laminate. It includes the results of bending tests on 'Araldite' glasscloth panels performed by the Royal Aircraft Establishment, Bedford. These tests show properties of laminates made with various 'Araldite' resins, glasscloth reinforcements and fillers. 'Araldite' epoxy resins, manufactured by Ciba (A.R.L.), Ltd., are extensively used in industry as adhesives, and for casting, laminating, tooling and surface coating. They were chosen by the Royal Aircraft Establishment, Bedford, for model-making because with glasscloth they give laminates with a very high strength/weight ratio. Several models of each type were required and the cold-laminating technique used resulted in these models being identical and of great dimensional accuracy. It is a feature of 'Araldite' epoxy resins that they cure with negligible shrinkage. A description of 'Araldite' and the uses to which it has been put at Bedford has been given by E. J. R. Thomas, of the Wood and Plastics Department (Ciba (A.R.L.). Ltd. Technical Notes No. 206 (February 1960): Model-Making with 'Araldite'. Pp. 12. Duxford: Ciba (A.R.L.), Ltd., 1960).

Measurement of Wind Speeds near a Tornado

An observation has been made of the speed of debris particles whirling round a tornado funnel from enlargements of the separate pictures of a cine film. The maximum value was 212 m.p.h. The observation was made in a tornado which struck Dallas, Texas, on April 2, 1957. On enlargement of the film at the Kansas City Research Unit of the U.S. Weather Bureau it proved possible to identify individual particles of debris whirling round the tornado and compute their tracks and speeds. The observations are reported in the October 1959 issue of the *Monthly Weather Review*.

The Neolithic Revolution

Recently, a British Museum (Natural History) publication entitled "The Neolithic Revolution" has appeared (Pp. vi+60+18 plates. London: British Museum (Natural History), 1959. 5*s*.). The author, Sonia Cole, is well known for her work on certain aspects of African prehistory. The discovery by early man of the possibilities of agriculture and the breeding of animals in captivity produced profound changes not only in his well-being, but also in his outlook on life. This neolithic revolution necessitated communal life, and for the first time man had to live close to his fellow man—not always an easy matter. Food could now be stored and a much larger population could survive. It is not too much to say that we can describe the prehistoric folk prior to these discoveries as our forerunners, those that come after them as our early ancestors. This well-illustrated work discusses such problems as the origins of grain cultivation, the domestication of animals—that is to say, breeding in captivity, not merely taming; flint mining and forest clearance; transport—particularly necessary when a further stage in civilization was reached and the village developed into the town; crafts and buildings.

Fishery Research Training Grants

The Development Commissioners have announced that, in association with the Ministry of Agriculture, Fisheries and Food and the Scottish Home Department, they will award postgraduate training grants in fishery research tenable from October 1. These training grants are intended to enable selected candidates to undergo a specified course of training to fit them for the investigation of problems in marine or freshwater science. Candidates must be British subjects and should be graduates with honours in science of a British university or possess equivalent qualifications. The value of the awards will be £420–£270 per annum depending on where the research training is carried out. Further information can be obtained from the Secretary, Development Commission, 3, Dean's Yard, Westminster, London, S.W.1. Applications should reach the Commissioners on or before May 20.

The British Textile Institute

The Textile Institute celebrates its golden jubilee this year, and in its awareness of the importance of science in the textile industry the theme of the annual conference will be "Fibre Science". The meeting will be held in London during May 30–June 3. The conference will be opened by Sir Harry Melville, who will speak on the impact of modern chemistry on industry in general. Other speakers will include Prof. J. B. Speakman (University of Leeds), Prof.

M. J. Lighthill (Royal Aircraft Establishment, Farnborough), the Rt. Hon. Douglas P. T. Jay, M.P., Prof. D. T. Jack (University of Durham), Prof. W. T. Astbury (University of Leeds), Prof. R. Meredith (Royal College of Science and Technology, Glasgow), Prof. R. Nicholson (Royal College of Art), Prof. Janey Ironside (Royal College of Art) and Sir Walter Puckey (chairman, Automation Consultants and Associates, Ltd., and of Management Selection, Ltd.). Further information can be obtained from the Golden Jubilee Conference Organizer, Textile Institute, 10 Blackfriars Street, Manchester 3.

Advisory Committee on Scientific Work of the Infestation Control Laboratory

THE Minister of Agriculture, Fisheries and Food has appointed the following committee to advise and report annually on the programme of experimental and research work of the Infestation Control Laboratory of the Ministry: Prof. J. B. Cragg, professor of zoology in the University of Durham (*chairman*); Mr. H. R. Hewer, reader in zoology, Imperial College of Science and Technology; Dr. T. Moran, director of research for the Research Association of British Flour Millers, St. Albans, Herts; Mr. F. Rayns, director of the Norfolk Agricultural Station, Sprowston, Norwich; Prof. H. G. Sanders, chief scientific adviser (agriculture), Ministry of Agriculture, Fisheries and Food; Prof. V. C. Wynne-Edwards, professor of natural history, University of Aberdeen. The secretary of the Committee is Miss Y. Larthe. Assessors to the Committee are: Mr. A. S. Fortune, chief inspector, Department of Agriculture for Scotland; Dr. R. A. E. Galley, director of the Tropical Products Institute, Department of Scientific and Industrial Research; Mr. G. V. B. Herford, director of the Pest Infestation Laboratory, Slough, Bucks; Mr. G. G. Samuel, Agricultural Research Council; Dr. E. B. Worthington, deputy director-general (scientific), Nature Conservancy.

Announcements

H.M. THE QUEEN has conferred the Order of Merit on Sir Cyril Hinshelwood, Dr. Lee's professor of chemistry in the University of Oxford and president of the Royal Society. Lord Radnor, chairman of the Forestry Commission since 1952, has been appointed a knight of the Order of the Garter.

DR. RICHARD O. ROBLIN, vice-president for research and development of the American Cyanamid Company, who is known for his work on sulphadiazine and sulphamerazine, has been elected president and director of the Cyanamid European Research Institute in Geneva; Dr. Mario Scalera, assistant to the general manager of the Central Research Division of Cyanamid, has been elected a vice-president of the Institute. The Institute, which last year acquired eight and a half acres of land for its laboratories in Geneva's Cologny section, will be devoted to long-range, basic research in the chemical and related fields.

THE Trustees of the Rockefeller Foundation have authorized a grant of 30,000 dollars to the Marine Biological Association of the United Kingdom for the purchase of research equipment for use in the Plymouth laboratory.

THE Latin American school of physics is to be held this year at the Centro Brasileiro de Pesquisas Físicas, under the auspices of Conselho Nacional de Pesquisas do Brasil, Academia Brasileira de Ciências, Campanha de Aperfeiçoamento de Pessoal de Nível Superior, Pan American Union and Unesco, during June 27–August 7. Further information can be obtained from J. Leite Lopes, Centro Brasileiro de Pesquisas Físicas, Av. Wenceslau Braz, 71 Rio de Janeiro, Brazil.

THE thirtieth annual report of the University Research Committee, University of Birmingham, gives a summary of departmental research progress during the academic year 1958–59. Lists of papers published during the year and of titles of those approved for higher degrees during the period are included in each departmental report, arranged alphabetically by authors (pp. 123. Birmingham: The University, 1960). A selected list of general works and further writings by members of the University staff is appended, arranged under faculties.

THE International Atomic Energy Agency is issuing a world list of institutions concerned with atomic energy (Gen./Pub. No. 3). The list is being issued at irregular intervals as a series of publications; each publication covering a Member State of the Agency. The list is intended to facilitate the exchange of information on nuclear energy between scientists, engineers, research workers and administrators in Member States. Copies can be obtained from the Scientific and Technical Information Division, International Atomic Energy Agency, Kärntner Ring, Vienna.

AN international conference on Powder Metallurgy is to be held at the Hotel Beltmore, New York City, during June 13–15. Further information can be obtained from Mr. Kempton H. Roll, Metal Powder Industries Federation, 60 East 42nd Street, New York 19.

THE sixth Commonwealth Mycological Conference will be held during July 15–22 at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1. Further information can be obtained from the Director, Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey.

THE thirteenth symposium in biology at the Brookhaven National Laboratory on Protein Structure and Function will be held during June 6–8. Further information can be obtained from Dr. D. E. Koshland, jun., Department of Biology, Brookhaven National Laboratory, Upton, L.I., New York.

THE second annual conference of the British Computer Society, Ltd., is being held during July 4–7 at the Sun Pavilion, Harrogate, Yorkshire. Further information can be obtained from Miss D. E. Pilling, Electronic Computing Laboratory, The University, Leeds 2.

THIS year the Charité (Faculty of Medicine), Humboldt University, Berlin, celebrates its 250th anniversary, and functions will be held in conjunction with the 150th anniversary of the University. Further information can be obtained from Dr. Dagobert Müller, secretary of the Committee for Preparations of the 250th anniversary of the Charité, Berlin, N.4, Schumannstrasse 20–21.

ERRATUM. In announcing the award of the J. Lawrence Smith Medal of the U.S. Academy of Sciences to Dr. E. J. Öpik (see *Nature*, April 23, p. 280), it was stated that Dr. Öpik is director of the Armagh Observatory; he is, in fact, a research associate of the Observatory, the director of which is Dr. E. M. Lindsay.

THE NATIONAL SCIENCE FOUNDATION, WASHINGTON

As in last year's report, rather more than half the ninth annual report of the (U.S.) National Science Foundation*, covering the year 1959, is occupied by appendixes listing the members of the National Science Board, its staff, committees and advisory panels, the grants for basic research and other purposes, grants for the International Geophysical Year programme, fellowship awards and publications, together with the financial report. About a quarter of the remainder deals with the re-appraisal and re-organization of the status of science in the United States, 1958–59, including a brief account of the two reports, "Strengthening American Science" and "Education for the Age of Science", coming from the President's Science Advisory Committee. This section, and the Chairman's foreword and the Director's statement, are discussed separately (see p. 341 of this issue); the remaining parts of the report, which review the activities of the Foundation itself, are reviewed here.

The significant increase in the support given by the Foundation to basic research in the United States from 25 million dollars in 1958 to almost 65 million dollars in 1959, should be noted first; this includes some 15·5 million dollars for research facilities, of which 12·3 million dollars were in support of facilities in mathematical, physical and engineering sciences. Support in the biological and medical sciences covers a wide range in developmental and environmental biology, systematic and regulatory biology. In genetic biology it is directed towards elucidating the nature of genetic material, the laws governing the transmission of hereditary traits, and the mechanisms controlling and determining the expression of hereditary characters; in metabolic biology, towards the mechanisms of protein synthesis and growth and specific metabolic factors which inhibit growth; in molecular biology, towards the investigation of the structure, synthesis and reactivity of peptides and proteins; and in psycho-biology, to the support of quantitative techniques in physiological and experimental psychology as these develop from mathematics and mathematical statistics.

In mathematical, physical and engineering sciences, a new programme for atmospheric sciences was established, under which the weather modification programme is now handled. Grants in chemistry were chiefly in organic and physical chemistry, while in the Earth sciences, support for oceanography was increased. In the engineering sciences, special reference is made to two grants in plasma dynamics, while the physics programme continued to place major emphasis on high-energy physics, particularly cosmic rays, and increasing attention was given to low-temperature research. Many of the facilities of the National Radio Astronomy Observatory are now approaching completion or actually in operation, and construction of the Kitt Peak National Observatory was started in 1959, where besides a 36-in. and an 84-in. reflector for stellar observations, it is proposed to instal a solar telescope with a parabolic mirror 60 in. in diameter and a focal length of 300 ft.

An Office of Social Sciences was established during the year to support research and related activities in social science disciplines, for which grants during the year totalled 853,000 dollars, including anthropological sciences, sociological sciences, economic sciences and the history and philosophy of science. The Antarctic Research Programme, which received 2·3 million dollars, is supported on a geographical basis and an interdepartmental committee has been set up by the Foundation to examine proposals and programmes of the various agencies and the broad programme suggested by the Committee on Polar Research of the U.S. National Academy of Sciences.

Among the significant research developments of the year to which the report directs attention are the sunspot photographs obtained with the *Stratoscope I* 12-in. balloon-borne solar telescope; the resolution of the galactic nucleus Sagittarius *A* with the new 85-ft. Tatel radio-telescope at Greenbank, West Virginia; the use of a new technique of testing for perception of visual depth to throw light on native and learned factors in the perception of distance; the synthesis of a pituitary hormone of 13 amino-acids linked in a specific sequence resembling adrenocorticotrophic hormone in structure; the identification of a catalytic factor from blood which can transform 1,000–10,000 times its own weight of soluble fibrin into the insoluble form; the discovery of a mollusc thought to be extinct for nearly 300 million years; the establishment of migrations of *Sequoia* from tropical swamps 60 million years ago through adaptation to drier and cooler climates; and the development of a tetrahedral anvil which can generate pressures up to 100,000 atm. at 3,000° C.

During the year the Foundation sponsored and provided partial support for 41 scientific conferences and symposia and awarded 419 grants, totalling 330,000 dollars, to enable scientists to attend international meetings. Fellowship awards totalled 3,937, amounting to about 13·1 million dollars, and with those assisted under the research grants programme, the number of scientists assisted during the year was about 6,000. Of the fellowships, 965 were in the biological and medical sciences, 2,857 in the physical sciences (chemistry, 730; geological sciences, 195; engineering sciences, 558; mathematical sciences, 689; physics, 663), and 59 in the social sciences. Of the total, 1,100 were pre-doctoral, 194 post-doctoral and 83 senior post-doctoral; 302 science faculty fellowships were awarded for improving the quality of science teaching, as well as 628 summer fellowships to secondary school teachers of science and mathematics, 580 summer fellowships for graduate teaching assistants, and 1,050 co-operative graduate fellowships of 2,200 dollars for 12 months. The plans for improving the quality of science teaching by assisting teachers in secondary schools to extend their knowledge of science and of new developments comprise four main programmes on which 33·25 million dollars were expended in 1959. The summer institutes programme provided stipends to

* National Science Foundation. Ninth Annual Report for the Fiscal Year ended June 30, 1959. Pp. xvii+274 (12 plates). (Washington, D.C.: Government Printing Office, 1960.) 1 dollar.

20,000 participants. Besides teachers in secondary schools and colleges attending 348 'courses' varying in length from 4 to 12 weeks, 515 teachers and supervisors in elementary schools received training in 12 summer schools; technical school personnel were provided for in two experimental summer programmes and 546 college teachers of science and mathematics attended 20 summer conferences dealing with special fields of science and mathematics. Under the Academic Year Institute programme, providing a year's study in science and mathematics for secondary school teachers, awards were made to 32 colleges and universities, and it is estimated that the 1959–60 programme will support more than 1,500 teachers; while opportunities for further study in out-of-school hours will be provided to about 9,000 secondary school teachers under the In-Service Institutes programme. Special projects in science education on which nearly 9 million dollars was expended cover visits of scientists to secondary schools, the provision of a travelling science library of carefully selected books for high schools, a travelling science demonstration lecture programme for secondary schools and support for summer training of secondary school students. The State Academies of Science, the College Programme and Teacher Improvement Programmes provide for visits by scientists, and foster the participation of undergraduates in research, supplementary training for science teachers and eight special field institutes covering advanced training in highly specialized subjects. On the Course Content Improvement Programme, a further 6 million dollars was expended on projects in elementary and secondary schools and in colleges; this included supplementary teaching aids such as films and television. Activities under the International Science Education Programme initiated during the year include the improvement of science curricula and the training of teachers and also measures to assist science students and scientists to engage in international educational programmes.

The Scientific Man-Power Programme, on which 780,000 dollars was expended in 1959, besides maintaining the National Register of Scientific and Technical Personnel, initiated surveys of scientific and technical employment in private industry and State Governments, of scientific and technical personnel in colleges and universities, and of scientists and engineers in Federal Government employment engaged in research, and pilot studies of the demand for scientists and engineers in several industries. Reports were completed on the knowledge of foreign languages of American scientists in 1954–55, on scientists and engineers in American industry in January 1957, and on employment and other characteristics of American scientific man-power during 1954–55. The measurement and appraisal of the national research and development effort is a major responsibility of the Foundation, and its Office of Special Studies has now completed its surveys of the year 1953–54; its present programme of statistical surveys proposes a detailed survey of each sector every four or five years, summary data only being collected in the intervening years. During the year, a survey was made of expenditure on scientific research and development in the fiscal years 1958, 1959 and 1960, and a survey of scientific research and development expenditure and man-power in colleges and universities during 1957–58 is now being conducted, following the publication of the report on the 1953–54 survey.

During the year, further responsibilities for improving the availability of research information to scientists were assigned to the Foundation by the National Defense Education Act, 1958, and accordingly the Office of Science Information Service and the Federal Advisory Committee on Scientific Information have been established. For the dissemination of scientific information, 146 grants totalling 3·8 million dollars were made for such purposes as documentation research, the international exchange of scientific information, the support of scientific publications and information services and the dissemination of unpublished research information such as unpublished Government research reports. Studies of the flow of information among scientists and of the scientific activity of chemists were completed during the year and five mechanical translation projects are at present supported, as well as the effort of 28 professional societies and academic institutions in providing translations of 35 Russian scientific and technical periodicals and 18 books and monographs. A Foreign Technical Information Centre has been established in the Office of Technical Services of the Department of Commerce, where scientific and technical translations prepared by Government agencies are collected, and their availability is announced. Support of scientific publications covered the issue of *Physical Review Letters*, an experiment in rapid low-cost publication of short, up-to-the-minute articles on physical research, support for the *Journal of Geophysical Research* and a grant to enable *Biological Abstracts* to extend its coverage; while grants in support of information services include one to the Bio-Sciences Information Exchange, administered by the Smithsonian Institution, and continued support to the Office of Critical Tables of the U.S. National Academy of Sciences.

THE PUBLIC AND THE BROADCASTING PROGRAMMES IN BRITAIN

THE British Broadcasting Corporation has issued a report on an inquiry carried out at the end of 1958 by its Audience Research Department*. Its first chapter describes the public and the facilities which are available for listening and viewing, comparing the public which depends solely on sound broadcasting with that which has television as well, and also contrasting viewers who can receive B.B.C. television only, with those who can also receive Independent television. The following three chapters deal successively with listening in the evening, viewing in the evening and listening in the day-time, contrasting the amount of listening or viewing, the selectiveness of listeners or viewers and the dis-

* British Broadcasting Corporation. The Public and the Programmes: an Audience Research Report on Listeners and Viewers, the time they devote to listening and viewing, the Services they patronize, their Selectiveness and their Tastes. Pp. 71. (London: British Broadcasting Corporation, 1959.) 8s. 6d.

tribution of their patronage. A fifth chapter discusses the tastes of listeners and viewers as they emerge when people have a chance to express their views in respect of a range of categories of programme output, and includes a comparison of the tastes of men and women of different age groups and different educational levels. Each chapter ends with a summary of its main findings, but a concluding section of the report entitled "Highlights and Reflections" attempts to view the results as a whole.

At the present time there are three distinct publics. The first, 'Band 1/3', which can receive both B.B.C. and Independent television and also sound broadcasting (now about half the population), is gradually swallowing up the other two: 'Band 1', which can receive B.B.C. television and sound (now about 20 per cent), and the 'sound only', which cannot receive television (now about 30 per cent). About 15 per cent had very high frequency receivers, and three-quarters of these are in television homes. The 'sound only' public is somewhat older and of a slightly higher educational level than the 'sound and television' public, and on average families in this group are a good deal smaller than 'sound and television' families. The 'Band 1' public also was somewhat older and of a rather higher educational standard than the 'Band 1/3' public.

The typical viewer watches television in winter for approaching two hours each evening, and this means that at peak hours not far short of half the adult population is watching television. The typical listener has his radio on for not much more than half an hour a night and will usually be doing something else at the same time. In television homes sound broadcasting is used very little in the evening, and after 6 p.m. it is catering primarily for people who have no television set. The vast majority of its day-time audiences, particularly in the early morning period and on Sundays, consists of people who have television sets. The inquiry showed that most people exercised their opportunity to choose between different programme services; those who did so, both viewers and listeners, outnumbering single-service loyalists by about two to one; but those who settled for one service were by no means homogeneous in either choice or tastes. There was an abnormally high proportion of discriminating listeners among those who limit their listening to the Home Service, and an abnormally high proportion of discriminating viewers among those who, though they could view Independent television, rarely did so.

A relation was noted between the amount of time people devote to listening or viewing and the services they patronize. The more time a person spends in listening the greater the proportion of that time he is likely to give to the Light Programme; similarly, the more he views, the greater is likely to be the proportion of his time spent in viewing Independent television. The inquiry showed that the less a person listens or views the more likely he is to be selective, and the more selective he is, the more likely he is to prefer the kinds of programme characteristic of the Home Service and the B.B.C. television than of the Light Programme and Independent television. Again, the older a listener is, the less likely he is to enjoy feature films, crime series, or westerns. Sex is sometimes predictive, women being more likely than men to be 'heavy' consumers of both sound and television in the evening. Science features on television, ranging sixteenth out of eighteen categories in popularity with the public, were not unpopular with men but were unpopular with women and most unpopular with viewers over sixty. Their popularity increases with each step up the educational level, and most often the individual's behaviour, attitudes and tastes tend to be associated with the age when his full-time education ceased, and/or 'occupational status', frequently reinforced by information about the newspapers he reads. Again and again the report demonstrates that the higher the individual's cultural level, the less he tends to listen or view, the more likely he is to be selective and to take an interest in serious types of programmes. Whatever measure of 'cultural level' is used, the population appears as a sharply tapering pyramid, and, accordingly, the more a type of broadcasting output or service depends for its custom upon people who have enjoyed cultural advantages or want something more than the undemanding relaxation, the more numerically restricted its audience is likely to be. So far as science is concerned the inquiry indicates that nearly all effective viewing is on B.B.C. television and hence anything that prejudices B.B.C. television as against Independent television would be liable to prejudice the use of television in the presentation of science to the public.

AUTOMATION AND ITS HUMAN REPERCUSSIONS

THE mental health problems of automation were examined recently by a World Health Organization study group (Study Group on the Mental Health Problems of Automation. (World Health Organization Report, Series No. 183.) Pp. 30. Geneva: W.H.O.; London: H.M. Stationery Office, 1959). It is not automation in the form of automatic co-ordination of machine tools on production lines, nor in the form of automatic control over manufacturing processes, that is liable to bring about the most revolutionary changes in mechanized work, but the rapid and automatic processing of an increasing range of technical and business information by the electronic digital computer.

Emotional reactions to automation range from unreasonable hopes of a rapid rise in living standards to fears of upheavals in conditions of employment and of difficulty in adaptation to the new machines. These reactions are of an anticipatory nature and are not based on actual experience. In the meantime, workers in automated undertakings are already suffering from physiological and psychological strain.

Because of its nature, automated work may have psychological consequences: for example, if the manual activity of a worker who has been accustomed to assess his work on the basis of his physical effort is reduced, he will feel himself less useful and suffer from a sense of inadequacy; if contact with the machine or with the object on which he is working is eliminated or reduced, he will see his task in a more abstract form and this may lead to anxiety; if his

responsibility is increased his job may become more of a strain.

What kind of strains are imposed upon the worker? First, there is the nervous strain caused by the need of unremitting attention to signals of varying perceptability, often separated by long intervals. The less the physical activity of such a worker and the greater the number of indicators to be watched, the more the nervous tension to which he will be exposed. In these circumstances particular importance will be attached to working conditions, such as temperature, sound-proofing, lighting, and the position of the operator and the machines. By preventing the operator from communicating with others, automated work tends to isolate him both physically and mentally. This feeling of isolation may be heightened by the background noise made by the machines.

In addition to its direct effects upon the workers' mental health, automation will undoubtedly have indirect social consequences. Families may, for example, be affected by the increased mobility of labour. The most serious difficulties indirectly affecting mental health are, however, liable to arise in connexion with the organization of the work itself. If automation calls for more shift work (as appears likely), this will certainly be opposed by the workers and their families.

Automation may provide certain satisfactions; for example, in an entirely automated plant, each operator becomes more or less his own master; the gap lessens between the factory worker and the office worker, and changes of occupation in middle age should be easier.

Efforts to mitigate the harmful effects of automation on mental health should be based on information and education. Education will be all the more effective if it is directed primarily towards those in key positions, that is to say, managers, engineers, trade-union leaders and the medical profession, particularly works doctors.

EMPLOYMENT OF WOMEN IN BRITAIN

THE Institute of Personnel Management has published under the title "Working Wives" a survey of facts and opinions concerning the gainful employment of married women in Britain carried out in co-operation with Mass Observation, Ltd., by Viola Klein during the autumn months of 1957, when out of 12,820,000 married women in Great Britain nearly 4 million were gainfully employed (Occasional Papers, No. 15. Pp. 63. London: Institute of Personnel Management, 1960. 7*s*. 6*d*.). Thus, in about one out of every three households the wife contributes by her earnings to the family income, but the apparent steep rise in the employment of married women since 1951 is due to the Population Census seriously underrating the number of married women in employment, particularly part-time. The outstanding impression gained by the survey is that women's lives to-day are dominated as much as ever by their role—actual or expected—as wives and mothers, and all other occupations are subordinated to their responsibility for home and family. The growth in the number of married women going out to work is due to the smaller size of their families and the general reduction in housework through the modernization of household techniques, combined with a striving to improve their standards of living. It is not widely assumed that married women should have jobs, although among the more highly educated single women in the sample nearly one third wished to continue their careers after marriage; this is nearly double the percentage in the next occupational group of women willing to continue in employment not merely temporary. The taking up of employment by married women appears to result from unforeseen though not unforeseeable circumstances, and men appear to be less conservative in their outlook in these matters than they are usually assumed to be.

Slightly more than one half the married women in employment have part-time jobs, which may be in the region of 4–30 hr. per week. The sample also showed that two-thirds of the women in part-time employment are engaged in unskilled or semi-skilled work, and that shorthand typing is the occupation least likely to offer any scope for the married woman who wishes to supplement her income by part-time employment. In some smaller occupational groups, such as teachers, nurses, social workers, a shortage of trained staff has opened the doors to part-time employees, and though the problems involved have not yet received the attention they deserve, more opportunities may well be offered here in the future for married women to combine a career with having a family. Women, in fact, it is concluded, are an essential and distinctive part of our man-power resources.

EXPLORATIONS OF THE GALÁPAGOS ISLANDS

THE Galápagos Islands have recently attracted widespread attention as a result of the Darwin centenary and the measures at present being taken to preserve and study their flora and fauna. The Galápagos Islands have been one of the principal fields of endeavour of the California Academy of Sciences since its first expedition there during 1905–6, and its collections from the archipelago are unsurpassed. It is therefore appropriate that the Academy should publish a short history of the islands by the late Joseph Richard Slevin, for more than fifty-three years associated with its Department of Herpetology, and a member of the original expedition, who for half a century maintained an active interest in the islands and published a number of scientific and popular papers about them (Occasional Papers of the California Academy of Sciences. No. 25: The Galápagos Islands: a History of their Exploration. By Joseph Richard Slevin. Pp. x+150. San Francisco: California Academy of Sciences, 1959).

This publication gives a description of the islands, their geology, climate, and fauna, but it is mainly

concerned with their history. An immense amount of literary research has gone into it, and the author has ransacked the archives of America and Europe to bring together the information that he has skilfully welded into a fascinating account of the islands from their discovery to the present day. He has consulted the original log-books of the early explorers, official reports from naval officers of many nationalities engaged on surveying, and the letters and logs of whalers and tortoise-oil hunters. There can be few records of visits to the islands that he has not seen, and his illustrations range from reproductions of Cowley's drawings made in 1684 to photographs of many interesting nineteenth-century ships that worked there, and of living giant tortoises and of the devastation of broken carapaces left by the oil hunters. The paper concludes with a notice of all the scientific expeditions that have been at the Galápagos, sections on the giant tortoises and the preservation of the fauna, and a select bibliography. Slevin could have wished for no better monument.

L. Harrison Matthews

THE AUSTRALIAN DEFENCE STANDARDS LABORATORIES

THE annual report of the Defence Standards Laboratories of the Department of Supply, Australian Defence Scientific Service, for the year ended June 30 1959, the staff of which numbered 621 at the end of the year, compared with 637 at July 1, 1958, includes lists of senior and professional staff, papers published, and committees on which the Laboratories' staff have served*. The Laboratories, besides providing a scientific advisory service to those concerned with development, manufacture, inspecting, procurement, storage and use of defence material, have a recognized function of research, both in relation to their scientific advisory function and in advising and assisting the defence services on the development of new and improved materials, methods and equipment. The range of the Laboratories' activities may be illustrated by mentioning a few of the investigations on which some notes are given in the report.

In chemistry these include studies of the pore structure and other physical characteristics of brown coal and copra charcoals, the oxidation of phosphorus in the decomposition of cast iron by acid, determination of minute amounts of ammonia by Nessler's reagent and of small amounts of tin, and of the dyeing of textiles. In metallurgy the study of chromium and chromium base high-temperature alloys continued, as well as of the production of pure chromium, the processing, properties and structure of chromium and chromium alloys and also on titanium alloys. In physics, further experience was gained in the technique of electron spin resonance, the use of X-ray diffraction in studying the structural changes in the ageing of titanium–chromium alloys, and on the stabilization of a.c.-line voltages. A stable electric arc was developed, and further work carried out on precise load measurement and the determination of gamma-ray source strengths. Miscellaneous work included improvements in electrophotography such as improved particulate photoconductor coatings of acceptance to charge on the surface, uniformity, sensitivity and spectral response, and the study of thin selenium films for xerography.

* Commonwealth of Australia. Annual Report of the Defence Standards Laboratories for the year ended 30th June, 1959. Pp. iv+75. (Maribyrnong, Vic.: Defence Standards Laboratories, 1959.)

HETEROGENEITY OF LECITHINS LABELLED WITH PHOSPHORUS-32

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SINCE the first use of phosphorus-32 in 1937 [1] for the study of phospholipid metabolism an immense amount of work has been done using this isotope; but in every case the results have been limited by the analytical methods used to separate the very complicated mixture of naturally occurring phospholipids. In the early work, for example, lecithin was isolated as the ethanol-soluble material, but this was far from pure. In more recent years the use of chromatography on alumina[2,3] or silicic acid[3] has yielded lecithins which analysed correctly in that the molar ratio of phosphorus : choline : fatty acid plus fatty aldehyde was equal to 1 : 1 : 2. In addition mild alkaline hydrolysis[4,5] of lecithin has been shown to yield glyceryl–phosphoryl–choline, which could be isolated and purified by paper chromatography. The use of these procedures enabled the mean specific radioactivity to be determined but took no account of the possible effects of the different fatty acids and fatty aldehydes that were present. Because of the absence of methods for fractionating the lecithins it has had to be assumed that any variation between lecithins was not large enough to invalidate comparisons of the specific radioactivities between the lecithins and other phospholipids. The following experiment shows that quite large variations in specific radioactivity can exist between different lecithins.

A rat (310 gm.) was injected intravenously with 0·8 mc. of labelled sodium dihydrogen phosphate in 0·1 *M* sodium chloride and 0·001 *M* sodium dihydrogen phosphate and killed after 90 min. The liver lipids were extracted, dinitrophenylated and methylated as described by me[6], and then divided at − 18° into an acetone-soluble and an -insoluble fraction. The acetone-insoluble materials were free of dinitrophenyl-containing lipids, and countercurrent distribution (120 transfers) in carbon tetrachloride–chloroform–methylene chloride–methanol–

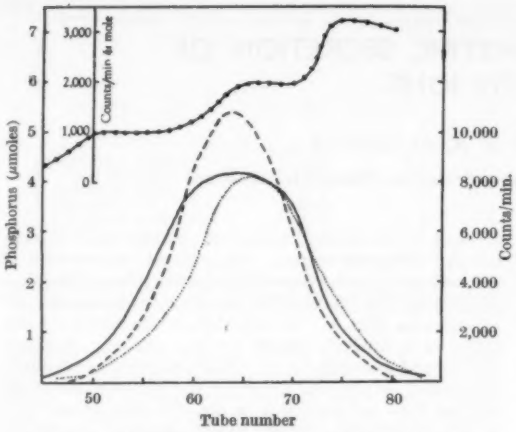

Fig. 1. Counter-current distribution of lecithin in carbon tetrachloride–methanol–water. —, phosphorus (μmoles); . . ., counts/min.; – – –, theoretical curve (phosphorus μmoles); · – · – ·, specific radioactivity counts/min./μmole

water (25 : 15 : 10 : 40 : 10 by volume) showed that lecithin (partition coefficient = 0·20) constituted 81 per cent of this fraction. The contents of the tubes containing the lecithin (mean specific radioactivity 1,750 counts/min./μmole) were combined and redistributed (120 transfers) in carbon tetrachloride–methanol–water (62 : 35 : 3·15 by volume) and the distribution obtained is shown in Fig. 1.

The theoretical distribution of 76 μmoles of a pure material with a partition coefficient of 1·15 is shown, and it will be seen that the experimental curve is broader and lower than that which would be expected for a pure compound. The curve for the radioactivity is skew, and the plot of the specific radioactivity shows a stepwise curve increasing in the more polar direction; the mean specific radioactivity is 1,690 counts/min./μmole but varied between 600 and 3,250 counts/min./μmole.

The lecithins were divided into three fractions obtained by combining the contents of the tubes as shown in Table 1. Each fraction was submitted to mild alkaline hydrolysis[4,5] and the water-soluble products subjected to paper chromatography in phenol–water (saturated). In each case a phosphate spot was detected[7] with an R_F of 0·90–0·95 which corresponded to glyceryl–phosphoryl–choline. The spot was cut out, extracted with water and the specific radioactivity of the phosphorus determined. The ether-soluble material remaining after the mild alkaline hydrolysis was further hydrolysed by heating with *N* potassium hydroxide in ethanol for 1 hr. The unsaponifiable material was extracted with diethyl ether from the alkaline digest and discarded. The mixture was then acidified and the fatty acids extracted with diethyl ether. The uptake of hydrogen of the fatty acids in the presence of a platinum catalyst was measured as an indication of the degree of unsaturation with the results shown in Table 1.

Table 1. Counter-current Distribution of Lecithin

After distribution in carbon tetrachloride–methanol–water the contents of the tubes shown below were combined and analysed

Fraction	*A*	*B*	*C*
Tube Nos.	44–59	60–70	71–83
Phosphorus (μmoles)	21·4	40·0	13·1
Specific radioactivity (counts/min./μmole phosphorus)			
Calculated from graph	1,000	1,750	2,520
From isolated glyceryl-phosphoryl-choline	985	1,930	2,750
Absorption of hydrogen (ml. at S.T.P.) per 100 mgm. fatty acids	6·89	9·14	Insufficient for analysis
Apparent 'partition coefficient'	0·85	1·22	1·73

There was close agreement between the specific radioactivity measured on the intact lecithin and the isolated glyceryl–phosphoryl–choline. Fraction *B* was more unsaturated than *A* but unfortunately there was insufficient material for the examination of fraction *C*. Synthetic dimyristoyl lecithin had a partition coefficient of 0·9 in carbon tetrachloride–methanol–water while the mid-points of fractions *A*, *B* and *C* correspond with partition coefficients of 0·85, 1·22 and 1·73 respectively. These results indicate that the saturated fatty acids predominate on the non-polar side of the distribution and the unsaturated ones on the more polar portion. Thus one can conclude that lecithins containing the more unsaturated fatty acids exchange more rapidly with inorganic-phosphate.

A preparation of a mixture of phosphatidyl ethanolamine and phosphatidyl serine (after dinitrophenylation and methylation) from the same animal as that used for the lecithin referred to above showed a similar skew distribution of radioactivity both in carbon tetrachloride–methanol–water (62 : 35 : 3·15 by volume) and in petroleum ether (b.p. 110–120°) : methanol. The specific radioactivity ranged from 10,600 to 16,500 counts/min./μmole phosphorus with the highest values on the more polar side of the distribution. The fatty acids were not examined and in this instance there was about 80 per cent phosphatidyl ethanolamine and 20 per cent phosphatidyl serine, but previous work had shown that there is little or no separation of these two phospholipids in the solvent system used. In spite of these limitations it is very likely that this skew distribution is also due to the partial segregation of phospholipids with differing fatty acid compositions.

The two examples given here show a range of specific radioactivity but their means still differ significantly. However, in other cases the results may not be so clear cut and some of the conclusions reached by the use of radiophosphorus may have to be revised. It will be necessary to fractionate each phospholipid in order to study the correlation between fatty acid composition and the turnover of the lipid-phosphorus.

Chromatography on silicic acid can be used[8] but counter-current distribution of the purified phospholipids provides a means in which the separations can be precisely controlled.

I am indebted to Dr. K. W. Zimmermann, Commonwealth Scientific and Industrial Research Organization, Melbourne, for the determination of hydrogen uptake of the fatty acids.

[1] Artom, C., Sargana, G., Perrier, C., Santangelo, M., and Segre, E., *Nature*, **139**, 1105 (1937).
[2] Lea, C. H., Rhodes, D. N., and Stoll, R. D., *Biochem. J.*, **60**, 353 (1955).
[3] Hanahan, D. J., Turner, M. B., and Jayko, M. E., *J. Biol. Chem.*, **192**, 623 (1951).
[4] Dawson, R. M. C., *Biochim. Biophys. Acta.*, **14**, 374 (1954).
[5] Benson, A. A., and Maruo, B., *Biochim. Biophys. Acta.*, **27**, 189 (1958).
[6] Collins, F. D., *Biochem. J.*, **72**, 281 (1959).
[7] Hanes, C. S., and Isherwood, F. A., *Nature*, **164**, 1107 (1949).
[8] Hanahan, D. J., Dittmer, J. C., and Warashina, E., *J. Biol. Chem.*, **228**, 685 (1957).

MECHANISM OF THE GASTRIC SECRETION OF HYDROGEN IONS

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THE experimental work and theories proposed for the formation of hydrochloric acid in the stomach were reviewed by Heinz and Öbrink[1]. Secretion of gastric acid appears to be dependent on aerobic metabolic processes; but glycolysis may also contribute to a small extent. The probable participation of various enzyme systems in the acid-producing mechanism was also discussed[1]. Such systems would eventually couple with the above-mentioned energy-yielding processes. Of the two ionic species involved, both the hydrogen and the chloride ions seem to be actively secreted, but the latter cannot be responsible for the essential part of acid formation[1,2]. Recently, Heinz and Durbin[3] accumulated more evidence for the existence of an independent 'hydrogen ion pump' in the stomach mucosa. However, at present there is no enzyme system that could be identified with any degree of certainty with this 'pump'.

The stimulating effect of histamine on the gastric secretion was first discovered by Popielski[4]. This effect is independent of the nervous system and, most probably, is due to a direct action of the drug upon the parietal cells of the gastric mucosa[5]. A steady concentration of histamine should be maintained in the body in order to achieve a steady state of elevated secretion of acid in the stomach (references in ref. 1).

Diphosphopyridine nucleotide (coenzyme I), which is an 'onium'-bearing compound, may undergo in the presence of a suitable enzyme[6] either hydrolysis or imidazolysis of its nicotinamide–ribose bond[7]. Both these processes are accompanied by the liberation of a hydrogen ion. The latter is, in a sense, forcibly ejected during the reaction, since both enzymic processes, namely, hydrolysis and imidazolysis, occur at neutrality, while protonation of either nicotinamide or the imidazolytic products of coenzyme I (for example, the histamine dinucleotide[8]) occurs at much lower *p*H values (for a thermodynamic evaluation of this question see ref. 9).

S-adenosyl-methionine, a sulphonium-bearing compound, may also undergo imidazolysis, as inferred from the *in vivo* work of Schayer *et al.* (references in ref. 10), a preliminary report of Brown *et al.*[11] and our unpublished studies (S. G. A. Alivisatos *et al.*, in preparation).

The above-mentioned observations led recently to the hypothesis that the immediate mechanism for hydrogen ion production in the stomach mucosa may involve the degradation of an 'onium' structure similar to that present in coenzyme I or in S-adenosyl-methionine. Hydrolysis or, alternatively, histaminolysis (imidazolysis) of this structure would yield the necessary hydrogen ions (Fig. 1). In view of the relatively large quantities of hydrogen ions secreted in the stomach, these processes should be reversible, provided that the required energy from aerobic or glycolytic metabolic sources would be available. Overall reversibility, however, would undoubtedly involve different routes. In general, restoration of the 'onium' structure would probably proceed through phosphorolytic pathways involving nucleoside triphosphates (Fig. 1). In addition, while direct reversibility in hydrolysis would be very unlikely, imidazolysis could possibly be to a limited extent reversible, depending on the *p*K′ value for the protonation of the imidazolytic product and on the *p*H range of activity of the enzyme(s). Such 'direct' reversibility could, perhaps, explain anomalies of 'back-diffusion' of hydrogen ions observed in histamine-stimulated stomach pouches (references in ref. 1).

According to this hypothesis, the stimulatory effect of histamine in gastric secretion would be due to its higher affinity, as compared to the affinity of water, for the enzyme-substrate intermediate. This would be in accord with the observation that, at neutrality, histaminolysis of coenzyme I predominates and proceeds to quantitative yields when the ratio of this imidazole to water is about one to 50 (ref. 12). By analogy with the intracellular distribution of the diphosphopyridine nucleotidase of rabbit erythrocytes[13], the enzyme(s) catalysing the reaction(s) postulated above may reside in the mucosal (secretory) side of the membrane of those cells of the stomach mucosa that are responsible for the hydrogen ion secretion (probably the parietal cells; however, see ref. 1).

The previously mentioned necessity of a steady histamine concentration in the body fluids as a prerequisite for a steady elevation of the hydrochloric acid secretion-rate in the stomach[1] pointed towards a perhaps small but continuous 'leak' of the hypothetical imidazolytic product. Accordingly, my first experimental approach was a trial to study the fate of histamine in the isolated stomach. It was hoped that this might lead to conclusions with respect to the nature of the postulated 'onium'-bearing structure. For this purpose, stomachs of white mice were excised and washed as described by Davenport *et al.*[14]. A glucose-containing Krebs–Henseleit type saline also described by Davenport[15] was used, after gassing with oxygen, for the washings and as an incubation medium. The washed stomachs were filled with this saline ('stomach contents', approximately 0·7 ml. per stomach) and both cardiac and pyloric ends were tied with silk thread. The liquid-inflated stomachs were then incubated at 37° C. with shaking, immersed in saline[15] ('nutrient medium', approximately 8·0 ml. per stomach). Throughout the incubation period of 60–90 min., a continuous stream of oxygen was bubbled through the 'nutrient medium'. In preliminary experiments it was found that under these conditions mice stomachs would produce about 3·7 μequiv. of acid per ml. per hr. When 2(ring) carbon-14-labelled histamine was included in the 'stomach contents' (4·0 μmoles per ml.; 4·75 curies per mole) acid production rose over that of the control (hista-

Table 1. Fractionation Scheme and Distribution of Radioactivity in Fractions obtained from Stomachs and Intestines of Experimental Animals after *in vitro* Incubation with a 2(ring) Carbon-14-labelled Histamine containing Saline

Specimen	I. Incubation medium	II. Organ contents (including organ walls)			
		(*a*) Material soluble in water : ethanol : ether (1 : 1 : 25)			(*b*) Material insoluble in water : ethanol : ether (1 : 1 : 25)
		1. Water	2. Ether	3. Insoluble	1. 0·2 *N* HCl fraction*
1. Stomachs, mouse	5·7	76·0	1·0	4·0	14·0
2. Stomachs, mouse	6·3	82·2	0·2	0·1	10·9
3. Stomachs, mouse	44·0	34·7	2·0	0·4	18·7
4. Stomachs, frog	45·0	44·5	0·2	0·1	10·2
5. Intestines, mouse	67·5	14·1	9·1	0·1	8·0
6. Intestines, frog	35·3	16·6	2·7	0·8	44·3

Each experiment comprised 4–5 stomachs or intestines. The figures represent per cent counts per min. of those originally added. Total radioactivity recovered in each experiment was about 95 per cent (mean value).
* The insoluble residue that could not be extracted with water (Fraction IIb_3) was not tested for radioactivity.

mine omitted) by about 30–50 per cent. Mouse intestinal fragments (ileum) were excised, washed and utilized in a similar manner. In experiments with frogs (*Rana pipiens* and *Bufo bufo bufo*) the saline[15] was diluted with water to 2/3 and incubations were carried out at room temperature. Frog stomachs (only the mucosal layers) were prepared as described by Davies[14]. Frog intestinal fragments (muscularis included) were prepared as above. At the end of the incubation, the 'nutrient medium' was acidified with 0·1 *N* hydrochloric acid to *p*H 2·0, while the liquid-inflated stomach (or intestine) was dropped in a mortar containing a dry-ice ether mixture. Three minutes later the ether was removed by decantation and kept aside, while the solid-frozen organ was pulverized with a pestle. A volume of ethanol equal to the volume of the 'contents' was then added to the moist powder and mixed. Afterwards, the previously used ether was recombined slowly under continuous stirring and the mixture was centrifuged at $1{\cdot}5 \times 10^3 g$ for 15 min., in the cold. The supernatant (water : alcohol : ether = 1 : 1 : 25) was evaporated to dryness under reduced pressure and the residue was extracted as indicated in Table 1 (*a*). The precipitate of this centrifugation (Table 1 (*b*)) was further extracted with 0·2 *N* hydrochloric acid. Attempts to solubilize the remaining residue by more drastic means have been, up to now, unsuccessful. Table 1 indicates the percentage radioactivity present in the various fractions. Evidently a considerable portion (5–6 per cent) of the total radioactive material penetrated through the stomach walls from the 'contents' towards the 'nutrient medium' (Nos. 1 and 2, Table 1). This material differed considerably in its migratory properties from histamine (1, Table 2). In experiments with frog stomachs (mucosal layer) and intestinal fragments (mouse or frog) increased physical porosity of the wall, often identifiable during the washings, contributed to the passage of larger quantities of unaltered histamine. This was also the case in a few experiments with mouse stomachs (Nos. 3–6, Table 1).

The various fractions of Table 1 were separately condensed to a small volume and used in paper chromatographic[12,17–19] and paper ionographic[8,9,12] studies. Results from an experiment with mouse stomachs are summarized in Table 2. They are expressed in terms of relative movements with respect to histamine. The migratory properties of a few imidazole derivatives are also included in Table 2. In general, during incubation with the stomach, a number of histamine derivatives were formed. In some instances as many as five distinct radioactive peaks could thus be registered (IIa_1, System *B*, Table 2). Although some of these peaks could represent artefacts formed during the fractionation procedures, notable differences observed between the products formed in the stomach and those in the intestine (not included in Table 2) strongly support the view that most of those peaks correspond to *bona fide* catabolic changes of the histamine molecule. Unchanged histamine was also present in most of the fractions, often representing the major peak (Table 2).

Ionographic studies at *p*H values ranging from 3·0 to 9·0 showed that cationic groups were predominant in almost every derivative. Many of these substances were devoid of any anionic group. How-

Table 2. Chromatographic and Ionographic Properties of Isotopically Labelled Substances present in Fractions obtained from Mouse Stomachs by the Procedure outlined in Table 1

Fraction (see Table 1)	Chromatographic systems*				Ionography† at *p*H 3·5
	A	*B*	*C*	*D*	
I	*1·35*‡ 0·34	*1·06*	*0·82* 0·37	*0·99*	*0·62*
IIa_1	*0·99* 0·61 0·42	*0·99* 0·68 0·55 0·40 0·28	*0·99* 0·55 0·39	*1·02* 0·78 0·63 0·52	*0·98* 0·73 0·61 0·49
IIa_2	1·66 *0·96* 0·50	*0·86* 0·43	1·04 0·96 *0·70* 0·42	*0·69* 0·46 0·29	*0·98* 0·45
IIb_1	*0·94* 0·64 0·28	*0·97* 0·72 0·34	*0·88* 0·38	*0·99* 0·73 0·41	*0·99* 0·61 0·03
Acetyl(side chain)-histamine	1·48	1·10	1·70	1·25	0·49
l-Histidine	0·59	1·44	0·27	0·65	0·45
Imidazole	1·37	1·34	1·86	1·20	0·90
Imidazole-(4 or 5)-acetic acid	0·79	0·23	0·35	0·89	0·22
N(ring)-methyl-histamine	1·15	1·07	0·98	1·07	0·98
2C^{14}-(ring) labelled histamine (R_F values)	0·53	0·62	0·44	0·68	1·00

* *A*, *n*-propanol/0·05 *M* acetic acid, 60/40 (ref. 12); *B*, *n*-butanol/ethanol/conc. ammonia, 80/10/30 (ref. 17); *C*, *iso*propanol/pyridine/water, 2/2/1 (ref. 18); *D*, pyridine/water, 65/35 (ref. 19). All proportions are volume per volume. Movements of the various substances were divided by the movement of histamine in the corresponding system, and the ratios (relative movements) are given in the table. These relative movements may be converted to R_F values by multiplying by the R_F values of histamine given above.
† Ionographic movements are expressed relative to the movement of histamine, the migration of which towards the negative pole was always taken as unity.
‡ Italic figures represent the major radioactive peak (up to 70 per cent of the total counts present in the fraction).

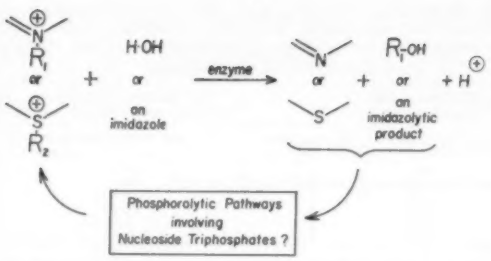

Fig. 1. Proposed mechanism of hydrogen ion production in the stomach mucosa. The postulated 'onium' structure could be an integral part of either a relatively small molecule or a large entity, possibly a lipoprotein embedded in the mosaic of the cell membrane (mucosal or secretory end). A similar intracellular distribution could also apply for the enzyme

ever, in a few instances anionic groups were present; but at most *p*H values they were effectively balanced or overpowered by the cationic groups present in the molecule. A limited number of ionographic experiments at high *p*H values (*p*H 11·5–12·5) indicated that N(ring)-substituted histamine derivatives might be included among these substances. Only results from experiments at *p*H 3·5 have been included as an example in Table 2. Further identifications based on migratory properties and certain colour reactions (diazo reaction, ninhydrin, etc.) would only be tentative at this stage and are deferred until isolation of each of these products, now in progress in this laboratory.

In conclusion, the experimental approach outlined above, namely incubation of isotopically labelled histamine in isolated stomachs and intestines of mice and frogs, led to the preliminary separation of a few derivatives of histamine. Isolation and identification of these derivatives are now in progress. It is hoped that this might lead to recognition of the moiety (R_1 or R_2 of Fig. 1) of the postulated substrate implicated in the hydrogen ion secretion. However, it should be emphasized that even after positive identification of this imidazolytic product, the exact nature of the parent compound bearing the 'onium' structure (Fig. 1) would still remain uncertain. Different approaches now considered in this laboratory would be required to clarify this problem.

I wish to thank my associate, Mrs. F. Ungar, as well as Messrs. D. Cohen and L. Lukacs, for their invaluable assistance in the performance of these experiments. This investigation was partly supported by Grant No. *E*-2436 from the U.S. National Institutes of Health, Grant No. *G*-7644 from the National Science Foundation, Washington, D.C., and Grant No. *P*-241 of the American Cancer Society, Inc.

[1] Heinz, E., and Öbrink, K. J., *Physiol. Rev.*, **34**, 643 (1954).
[2] Durbin, R. P., and Heinz, E., *J. Gen. Physiol.*, **41**, 1035 (1958).
[3] Heinz, E., and Durbin, R., *Biochim. et Biophys. Acta*, **31**, 246 (1959).
[4] Popielski, L., *Pflug. Arch. ges. Physiol.*, **178**, 214 (1920).
[5] Code, C. F., in "Histamine". Ciba Foundation Symposium ed. by Wolstenholme, G. E. W., and O'Connor, C. M., 191 (Little, Brown and Co., Boston, 1956).
[6] Alivisatos, S. G. A., and Woolley, D. W., *J. Biol. Chem.*, **219**, 823 (1956).
[7] Alivisatos, S. G. A., *Nature*, **183**, 1034 (1959).
[8] Alivisatos, S. G. A., *Nature*, **181**, 271 (1958).
[9] Alivisatos, S. G. A., La Mantia, L., Ungar, F., and Savich, B., *Biochim. et Biophys. Acta*, **33**, 660 (1958).
[10] Schayer, R. W., *Physiol. Rev.*, **39**, 116 (1959).
[11] Brown, D. D., Axelrod, J., and Tomchick, R., *Nature*, **183**, 680 (1959).
[12] Alivisatos, S. G. A., Ungar, F., Lukacs, L., and La Mantia, L., *J. Biol. Chem.* (in the press).
[13] Alivisatos, S. G. A., and Denstedt, O. F., *Science*, **114**, 281 (1951).
[14] Davenport, H. W., and Jensen, V., *Gastroenterology*, **11**, 227 (1948).
[15] Davenport, H. W., "In Methods in Medical Research", ed. by Visscher, M. B., **4**, 185 (The Year Book Publishers, Inc., Chicago, 1951).
[16] Davies, R. E., *Biochem. J.*, **42**, 609 (1942).
[17] Schayer, R. W., Wu, Kwang Ying Tai, Smiley, R. L., and Kobayashi Yutaka, *J. Biol. Chem.*, **210**, 259 (1954).
[18] Karjala, S. A., and Turnquest, III, B. W., *J. Amer. Chem. Soc.*, **77**, 6358 (1955).
[19] Karjala, S. A., *J. Amer. Chem. Soc.*, **77**, 504 (1955).

SIGNIFICANCE OF THE 'EQUIVALENT REDOX POTENTIAL' IN AQUEOUS SOLUTIONS UNDER IONIZING RADIATION

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In a recent paper[1], Henderson, Lovering, Haines and Casey presented results which support the view of Dainton and Collinson[2] that an aqueous system exposed to ionizing radiation has a unique 'equivalent redox potential' which is characteristic of the solvent. This concept arose from Allen's prior discussion of the fate of an added oxidation-reduction couple under irradiation[3]. The concept was criticized by Veselovsky[4], who pointed out briefly that it neglects consideration of the kinetic factors involved. Reference made to work done by Clark in Oak Ridge National Laboratory[5,6] indicates that the kinetic factors still have not been adequately appreciated, though the experimental results of Henderson *et al.* appear to be entirely valid and constitute an excellent confirmation of the general idea.

To discuss the equivalent redox potential requires careful discrimination as to the nature of the potential under consideration. The radiolytic radicals, for example, H and OH, are not in equilibrium with water, but are present at concentrations dependent upon the intensity of irradiation and the rates of their disappearance by various processes. If one knew the standard free energies of H and OH and their concentrations in the irradiated solution, one could calculate the e.m.f. of a cell corresponding to the overall reaction $H + OH \rightarrow H_2O$. Such an e.m.f. could be measured only if it were physically possible to have two electrodes, each acting reversibly with respect to one of the half-reactions alone. This e.m.f. would bear no relation to a single potential such as the equivalent redox potential is considered to be, however. To refer to a real and presumably measurable half-cell potential characteristic of the aqueous medium under irradiation presupposes the insertion of an inert conducting material for the

measurement of the potential. When this is done, however, the potential measured between the electrode and a reference electrode is a steady-state mixed potential, the value of which depends upon the kinetics of all processes, anodic and cathodic, occurring at the interphase between metal and solution[7]. The observed potential will depend upon electrode reactions by which the system is attempting to approach a state of chemical equilibrium while radicals are being continuously produced, and the rates of these processes are determined by kinetic parameters. Posey[8] has given a mathematical treatment of similar situations, which would be applicable here if the electrode processes were under activation control and the requisite data were available.

The measurable, although irreversible, value of an electrode potential in an irradiated solution thus includes anything that affects the polarization of the electrode, such as the activation energies of the individual processes, which, in turn, depend in different ways upon the composition of the electrode and its surface state. Such effects were shown clearly by the experiments in this Laboratory and also in those cited by Veselovsky. The potential is affected also by the passage of different gases through the solution, or by addition of materials which react rapidly with one or the other of the active radicals produced by radiolysis. Initial reaction-rates are chiefly interesting to radiation chemists in connexion with the determination of G values. These are necessarily complicated, however, by the fact that the radiolytic products may act both as oxidizing agents and as reducing agents in individual cases, and that the oxidation-reduction reactions may proceed through unstable intermediates. There are numerous competing processes, therefore, and an electrochemical treatment is scarcely possible, though transient potentials are conceivable which would still be determined by the particular mechanisms in operation and by the condition that the sum of all anodic currents at the electrode must equal the sum of all cathodic currents on open circuit. The kinetics of such processes has been determined in certain cases[9]. The discussion which follows applies only to steady states.

We may consider, first, the case in which the solution contains no redox system other than radiolytic radicals themselves and their reaction products, at concentrations which may depend upon the type as well as the intensity of radiation. At an electrode, these reactants enter into electrode reactions in the futile attempt to approach equilibrium, the direction of change in each reaction being in accordance with the appropriate free energies. The electrode then takes a potential that is fixed uniquely by the kinetics of all cathodic and anodic processes occurring on it. Clark's measurements were made in a solution containing no redox couple foreign to the water and acid, other than trace impurities. With a platinized electrode in 0·10 N sulphuric acid at 85° under gamma radiation and exposed to air, the stable potential was found to be approximately 0·90 V. noble to a reversible hydrogen electrode in the same solution. The potential was shown to be quite different, however, when the solution was swept by hydrogen or nitrogen, or when smooth platinum or stainless steel replaced the platinized electrode. In these measurements, the potential was determined by electrode reactions of radiolytic products alone, and the value shifted rapidly upon application or removal of the gamma source.

If, now, a redox system is added to the solution after the steady-state potential appropriate to radiolytic products alone has been established, additional electrode processes become possible. Assume first that reactions of the added substances with radiolytic products occur only heterogeneously as electrode processes at the surface of the electrode. Depending upon the value of the normal potential of the redox couple, the activity of its components in the solution and various kinetic parameters, these reactions will polarize the electrode either cathodically or anodically, the relative activities of the oxidized and reduced species changing correspondingly. Since radiolytic products are being formed continuously, however, no steady-state potential can be attained until net oxidation or reduction of the added components proceeds to the point that their potential at the same electrode equals the potential of the radiolytic products alone under the prevailing experimental conditions. (This assumes that the processes at the electrode are kinetically independent.) Hence the observed potential should be independent of the system added, so long as a true steady state is reached. Unless the E^0 value of the oxidation-reduction couple is within a few tenths of a volt of the potential indicated by the platinized (reversible) electrode, however, one or the other of its components must necessarily be converted essentially completely into the other, as observed. If the reactions occurred only at the electrode, however, the fate of the redox couple would depend upon the polarization characteristics of the electrode used.

The situation is not greatly different if it is assumed, more realistically, that the reactions between added components and radiolytic products proceed chiefly and rapidly in the homogeneous aqueous phase. Again, the steady state to be reached involves production and consumption of radiolytic products at equal rates. The oxidation-reduction reactants then adjust themselves to such activities that this condition is satisfied. In other words, the relative activities of the added oxidation-reduction components in the steady state have become such that their reactions no longer disturb the steady-state concentrations of radiolytic products attained in the absence of these components. A suitable indicator electrode inserted in the solution will now give the corresponding redox potential, which the results of Henderson *et al.* show to be essentially independent of the identity of the system used. That a platinized electrode in Clark's experiments without added oxidation-reduction components indicated nearly the same potential as observed by Henderson *et al.* at a lower temperature shows merely that this type of electrode indicates reliably the kinetics of the radical reactions in the homogeneous phase, which is not true for stainless steel or silver, for example.

The behaviour of the potential upon cessation of irradiation is also understandable. In Clark's experiments, no added oxidation-reduction system was present other than trace impurities; hence the potentials shifted rapidly when irradiation ceased. The shift was in the direction determined by the transient condition then prevailing at the active interphase. In the experiments of Henderson *et al.* cessation of irradiation left an oxidation-reduction system in solution in electrochemical equilibrium, with concentrations much larger than those of the

radiolytic products by which the steady-state potential was determined in their absence. The potential therefore shifted only slowly from the reversible potential of the couple at the existing activities, doubtless owing to reactions of residual radiolytic products of long life, such as hydrogen peroxide.

Dainton and Collinson discussed the numerical value of the equivalent redox potential in terms of reversible couple potentials for H and OH, respectively. Since the equivalent redox potential is a kinetic, rather than a thermodynamic quantity, however, the free energies determined the direction of the several reactions, but not their rates, or the value of the resulting mixed potential. Clark observed that a platinum electrode in a solution swept by hydrogen showed no detectable change in potential when the gamma source was applied. This demonstrates that, at 85°, the electrode processes were unaffected by the hydrogen atoms and hydroxyl radicals produced, or, in other words, that the normal exchange current of the process $(Pt)H_2 \rightleftharpoons 2H^+ + 2e^-$ was sufficient to prevent a change of potential by any added electrode reactions due to hydrogen atoms or other active species formed under irradiation. According to Veselovsky, the normal potential of the hydrogen electrode is almost realized when an acidic solution is irradiated after being freed of dissolved gases. These results agree in indicating that hydrogen atoms disappear so rapidly in one way or another that they do not disturb the equilibrium among H_2, H, H^+, and electrons at the metal-solution interface.

Removal of hydrogen atoms as a chief reactant still does not explain the observed potential, however, since the reversible normal potential of such a couple as $OH + H^+ + e^- \rightarrow H_2O$ would be approximately 3 V. noble to a hydrogen electrode in the same solution. It is therefore evident on electrochemical grounds that OH radicals also disappear rapidly. It is to be observed, further, that both the value of the equivalent redox potential in the presence of air or radiolytic oxygen and its dependence on *p*H are approximately the same as those indicated by a platinum electrode in a dilute solution of hydrogen peroxide[10]. From these observations it appears that, although short-lived atoms and radicals may participate in both homogeneous and electrode reactions, the principal electrochemical effects may be ascribed to molecular hydrogen and oxygen and hydrogen peroxide. The mixed potential indicated by any given electrode therefore depends upon both the polarization characteristics of the electrode, including its surface state, and also any conditions that alter the surface concentrations of these radiolytic products. The value 0·85–0·90 V. noble to the hydrogen electrode is to be considered a unique property of the system only when a reversible electrode is used and when air is present or radiolytic products are allowed to accumulate in a steady state. The potential is independent of the identity of the redox system, so long as a steady state is reached ; but its measurement is facilitated by having such a system present to give stability to the potential.

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[1] Henderson, H. S., Lovering, E. G., Haines, R. L., and Casey, E. J., *Canad. J. Chem.*, **37**, 164 (1959).
[2] Dainton, F. S., and Collinson, E., "Ann. Rev. Phys. Chem.", **2**, 99 (1951). Cf. also *Faraday Soc. Disc.*, **12**, 251 (1952).
[3] Allen, A. O., *J. Phys. Chem.*, **52**, 479 (1948).
[4] Veselovsky, V. I., Int. Conf. Peaceful Uses of Atomic Energy, **7**, 599 (1956).
[5] Clark, W. E., and Cartledge, G. H., Oak Ridge Nat. Lab., Semi-Annual Prog. Rep., Chemistry Div., June 20, 1956, *ORNL*-2159, p. 71.
[6] Clark, W. E., *J. Electrochem. Soc.*, **105**, 483 (1958).
[7] Cartledge, G. H., *Werkstoffe und Korrosion*, **9**, 493 (1958).
[8] Posey, Franz A., *J. Electrochem. Soc.*, **106**, 571 (1959).
[9] See, for examples, Boyle, J. W., Weiner, S., and Hochanadel, C. J., *J. Phys. Chem.*, **63**, 892 (1959).
[10] Bockris, J. O'M., and Oldfield, L. F., *Trans. Farad. Soc.*, **51**, 249 (1955). Cf. also Gerischer, R., and Gerischer, H., *Z. phys. Chem.*, **6**, 178 (1956).

STEROL ANALYSIS BY GAS CHROMATOGRAPHY

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USE of gas chromatography in the analysis of sterol mixtures by itself or as a supplement to other methods of separation may yield more detailed results than have been possible up to now. The relative simplicity with which in general very effective separations can be rapidly obtained—in which case direct quantitative determination is frequently possible—is a great advantage of this technique.

We have investigated the possibilities of the analysis of sterols using model substances and natural mixtures. In view of the very low volatility of sterols, high column temperatures had to be used ; but in the high-temperature range the choice of the stationary phase is considerably limited.

'Apiezon *L*' and alkathene appeared to be completely unsuitable for this purpose. The sterols eluted as broad, asymmetric peaks. With silicon oil, on the other hand, completely symmetrical peaks were obtained.

We used the residue of a molecular distillation at 200° C. and 2μ pressure of Midland silicones No. 550. The carrier, 'Celite 545', was treated with acid to remove traces of metal, screened to a suitable particle size and treated with dichloro-dimethyl silane as described for 'Hyflo'[1] to make it as inert as possible.

At present we have only investigated separations on a relatively short column (length : 90 cm. ; internal diameter : 4 mm.) packed with 20 per cent by weight of silicone oil on 'Celite'. The best results were obtained with 'Celite' having a particle size of 150–178μ. The column was conditioned at a working temperature (287° C.) for 2 days. During this period the column was repeatedly vibrated and filled up with packing until a constant level had been obtained. The substances were brought on to the column as such in a small perforated stainless steel basket, or dissolved in methyl myristate using a capillary pipette. As detector the gas density balance[2] was

used, which, together with the column, was placed in an electrically heated air-bath. With the help of the internal standard technique, it was found that the elution of the investigated substances proceeded quantitatively.

Infra-red recordings of cholesterol, before and after passing the column, displayed no differences. It is evident, therefore, that no structural changes occur at this high temperature.

Fig. 1 shows the separation of cholesterol, 7-dehydrocholesterol, ergosterol and stigmasterol. Because of the conjugation in the *B*-ring of 7-dehydrocholesterol there is such a difference in volatility with cholesterol that these two substances can be separated almost completely. The influence of the length of the side-chain upon the retention time appears from the separation of ergosterol, stigmasterol and cholesterol. Cholesterol was also completely separated from technical sitosterol (supplied by Messrs. S. A. F. Hoffmann-La Roche and Co., Ltd., C.O. Basel). The sitosterol, however, was found to consist of at least three components. Separations can also be effected of sterol esters (Fig. 2).

The following example shows that with gas-chromatography it is possible to obtain separations which can only be indirectly achieved when other methods are applied.

When feeding rats a fat-free diet the fæces contain cholesterol, cholestanol, Δ^7-cholestenol, 7-dehydrocholesterol, coprostanol, and methostenol. The analyses of these sterols have been worked out by Coleman, Wells and Baumann[3,4]. Application of their chromatographic methods results in the separation of two main sterol bands, the so-called zones *C* and *D*. Zone *D* contains at least two sterols: coprostanol and methostenol. These two substances can be determined individually by means of the Liebermann–Burchard reaction. Coprostanol is the slow- and methostenol the fast-acting component. The structure of methostenol appeared to be: 4 α-methyl-Δ^7-cholesten-3 β-ol[5].

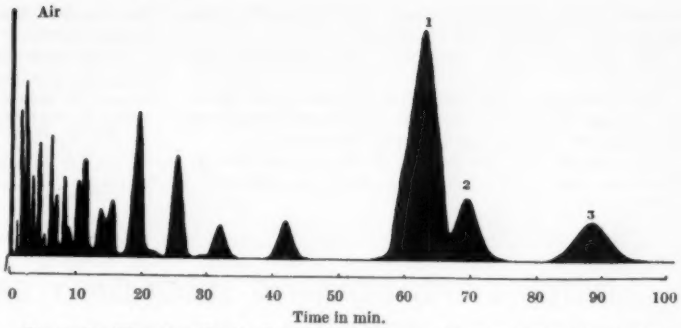

Fig. 3. Conditions (see Fig. 1). Zone *D* from rat fæces: (1) coprostanol; (2) probably cholestanol; (3) methostenol

Coprostanol and methostenol can only be separated chromatographically via the *p*-phenyl azobenzoates, which is a time-consuming process. By means of gas-chromatography, however, these sterols can be rapidly and quantitatively separated and determined.

Fig. 3 shows the chromatogram of the above-mentioned band *D* from the unsaponifiable fraction of rat fæces. The peaks in the sterol range are indicated as 1, 2 and 3. These three substances were collected individually and their behaviour with respect to the Liebermann–Burchard reagent investigated. Coprostanol, the slow-acting sterol, is represented by peak 1, methostenol as fast-acting sterol by peak 3.

The substance in peak 2, with a retention time equal to that of cholestanol and cholesterol, gave a negative Liebermann–Burchard reaction. On application of a modified chromatographic procedure based on the method of Coleman *et al.*[4] zones *C* and *D* can be eluted. This proceeds in such a way that on elution of the bands zone *C* leaves the column immediately after zone *D*. Cholestanol is already present at the beginning of band *C* and slight overlapping of the two zones is possible. Cholestanol also gives a negative Liebermann–Burchard reaction and therefore it is highly probable that the substance in peak 2 is cholestanol originating from zone *C*.

The gas-chromatographic separation obtained illustrates the great influence of methyl addition in the *A*-ring of the cholestenol molecule upon the retention time. At the same time it appears that the stereoisomers cholestanol and coprostanol are completely separated.

Table 1 contains the relative retentions of the sterols mentioned as well as of some higher hydrocarbons.

From our results it appears that sterols which differ in the total number of carbon atoms can not only be readily separated but can also be determined

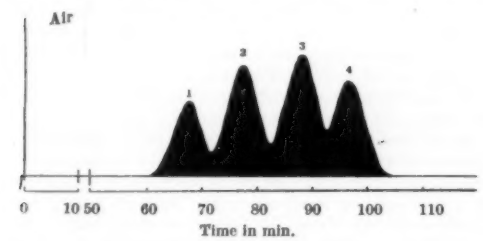

Fig. 1. Conditions: column, 90 cm. × 4 mm.; packing, 'Celite' 150–178μ; silicon oil, 4:1; temperature, 287° C.; overpressure on top of the column, 53 cm. mercury; flow-rate, 24 ml. N_2/min. Separation of: (1) cholesterol; (2) 7-dehydrocholesterol; (3) ergosterol; (4) stigmasterol

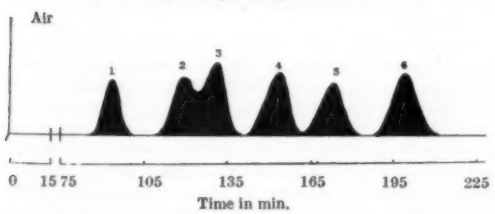

Fig. 2. Conditions (see Fig. 1). Separations of esters of cholesterol: (1) acetate; (2) propionate; (3) isobutyrate; (4) butyrate; (5) isovalerate; (6) valerate

Table 1. Retention Volume relative to Cholesterol on Silicon Oil

Coprostanol	0·9	Cholesterylacetate	1·42
Cholesterol	1	Tetratriacontane	1·45
Cholestanol	1	Cholesterylpropionate	1·83
Δ^7-Cholestenol	1·14	Cholesterylisobutyrate	2·0
7-Dehydrocholesterol	1·14	Cholesteryibutyrate	2·32
Methostenol	1·28	Cholesterylisovalerate	2·64
Ergosterol	1·30	Cholesterylvalerate	3·02
Stigmasterol	1·36	Octatriacontane	3·96

quantitatively, and if necessary the various fractions be used for further identification.

The plate number computed for cholesterol was 2300.

The resolution obtained can be improved by applying longer packed or coated capillary columns in combination with more sensitive detectors.

Our thanks are due to Mr. P. Smits and Mr. H. Dronkers, who carried out the group separation of the sterols. Further, we are greatly indebted to the management of Unilever N.V. for permission to publish these results.

[1] Martin, A. J. P., and James, A. T., *Biochem. J.*, **63**, 138 (1956).
[2] Howard, G. A., and Martin, A. J. P., *Biochem. J.*, **46**, 532 (1950).
[3] Wells, W. W., Coleman, D. L., and Baumann, C. A., *Arch. Biochem. Biophys.*, **57**, 437 (1955).
[4] Coleman, D. L., Wells, W. W., and Baumann, C. A., *Arch. Biochem. Biophys.*, **60**, 412 (1956).
[5] Neiderhiser, D. N., and Wells, W. W., *Arch. Biochem. Biophys.*, **81**, 300 (1959).

DETOXICATION DURING SECONDARY GROWTH IN PLANTS

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PLANTS, in contrast with animals, do not possess well-defined organs for the excretion of potentially injurious metabolic products. However, it is obvious that means must exist whereby toxic substances are rendered non-toxic or, if toxic, are 'excreted' to locations within the plant where the toxicity of the substances is of little consequence. For example, it is well known that succinic and oxalic acids are immobilized, in special parenchyma cells of secondary xylary tissues, as crystals of aluminium succinate and calcium oxalate.

In general, substances with reactive groups (whether they be toxic or non-toxic) are transported usually in an inactivated state to the regions of active secondary growth. For example, sugars are transported chiefly as sucrose, coniferyl and sinapyl alcohols as glycosides, and so on. These products of photosynthesis are soluble and, for purposes of permanent or temporary storage, they are converted, enzymatically, to the polymolecular ergastic substances of the cell wall (cellulosic and non-cellulosic polysaccharides and lignin) and the materials extraneous to the cell wall (tannins, kinos, lipids, starch, etc.).

High concentrations of acids and especially of phenolic substances exhibit an inhibitory effect or are toxic to cambial tissues. For example, Jacquiot[1] has shown that tannins inhibit the proliferation of isolated fragments of cambial tissues from *Quercus* and *Castanea*. In agreement with this observation, it has been found, for *Eucalyptus regnans*, that tannins are present in minimal amounts in the cambial zone and that the amounts increase on each side of the cambium; this evidence was taken as an indication that tannins are waste materials which are excreted to each side of the cambium and eventually accumulated in the tanniniferous parenchyma cells of the phloem (eventually transformed to the rytidome and thus periodically shed), and, via the parenchyma cells of the sapwood, in the dead cells of the heartwood[2]. Thus the formation of tannins and their excretion as outlined above may be, primarily, a means of 'detoxication' of excess phenolic substances. This does not mean that the tannin precursors (possibly by-products formed during the biosynthesis of lignin[3] or flavonoid compounds[3]) or the tannins themselves may not have important physiological functions in plant life; for example, it has been suggested that they may act as antioxidants or as inhibitors of fungal growth[4].

Again, lignin is a polymer formed from phenolic base units. Hence lignification, whether it proceeds by the utilization of phenolic substances from the nutrient phloem 'sap' stream ('detoxicated' coniferyl or sinapyl alcohols[5], flavonoid compounds[6], etc.) or by the utilization of lignin precursors produced within the developing cell either during its normal active life[7] or during its senescence[8], may be essentially a process of 'detoxication'. A secondary 'evolutionary' function of lignin may be to give rigidity and compressive strength to the cell walls of xylary and other plant tissues; however, plant tissues tend to be brittle when the lignin content is high[4,9].

There is much indirect evidence which indicates that, during lignification, the lignin becomes chemically bonded to the non-cellulosic polysaccharides of the primary, and outer portions of the secondary, cell walls. Much of the evidence suggests that the lignin is attached, by ester bonds, to the uronic acid units. For example, it is known that ruthenium red, a stain specific to carboxyl groups[10] in mature fibre cell walls, stains the intercellular layer and primary cell wall more intensely than any other portion of the cell wall; in the case of *Eucalyptus regnans* wood, the intensity of staining diminishes as lignification proceeds (Wardrop, A. B., unpublished work) and becomes almost zero in the mature fibre cell wall; analogous staining behaviour has been observed during the lignification of *Pinus sylvestris*[11]. When sections of mature wood of *E. regnans* are hydrolysed with dilute acids or alkalis, the red-staining potentialities increase with time of hydrolysis, the alkali being about 10^3 times as effective as acid in this respect (Stewart, C. M., Watson, A. J., Dadswell, H. E., and Kottek, J. F., unpublished work). Also, when holocellulose, prepared from *E. regnans* wood, is treated with dilute alkali (0·08 per cent sodium hydroxide for 1 hr. at 98°) the residual lignin is removed[12].

Many workers have shown that the 4-O-methyl-D-glucuronic acid units of the non-cellulosic polysaccharides of woods are attached glycosidically to the xylose units of the polymer chains. Thus, during lignification, lignin units may become attached to the uronic acid residues by the formation of 'lignyl' 4-O-methyl-D-glucuronate side-chains linked through the 2-O- or 3-O-positions on to the xylose units of the main chains of the glycosan uronides; this linkage process, and the possible further addition of lignin units to those already incorporated in the side-chains,

may represent the 'detoxication' mechanism which is applicable to the plant cell wall. During animal metabolism, many aromatic substances appear in the urine as derivatives of D-glucuronic acid; for example, alcohols and phenols appear as β-glycosides (for example, phenyl β-D-glucosiduronic acid) and carboxylic acids appear as β-hemiacylals (for example, benzoyl β-D-glucosiduronic acid)[13]. Hence uronic acids may serve as 'detoxication' agents during the metabolism of both plants and animals.

Perhaps other examples of 'detoxication' during the secondary growth of plants could be cited. For example, excess aliphatic acids such as formic and acetic acids, which do not form insoluble salts, may be immobilized by their attachment, as formyl or acetyl groups, to certain of the cell-wall polysaccharides; glycosan uronides, especially in hardwoods, have relatively high contents of acetyl groups, equivalent in some cases to about 25 per cent acetylation[12]. Again, alcohols may be immobilized as O-alkyl or alkoxy groups attached either to cell-wall components or to extraneous substances.

From the above discussion it will be noted that there are several possible general means of 'detoxication' and/or excretion which may operate within a plant. Normally, while in solution, the toxic substance is 'detoxicated' by the formation of a non-toxic derivative, usually a glycoside. If the production of such substances were to continue, without some means of lowering their concentration, the cell fluids would become extremely viscous because of the high concentration of substances in solution. A mechanism of preventing the formation of high concentrations must exist; this mechanism, in general, seems to depend upon polymerization of the soluble substances to ensure temporary or permanent 'detoxication' of reactive toxic groups (during the polymerization reactions), with parallel increased insolubility and deposition in the walls or cellular contents of cells, which will eventually be isolated from the regions of active growth.

In the case of substances such as aliphatic acids which neither form glycosides nor readily polymerize, two means of 'detoxication' seem possible: first, the formation of insoluble salts which are stored in special crystalliferous parenchyma cells; and, secondly, attachment as acyl groups to the glycosan uronides, excess acids perhaps being excreted to the bark or heartwood. It should be noted, especially in old xylary tissues, that the acids may be re-liberated from acyl groups by autocatalysis, with consequent damage to the tissues resulting from prolonged hydrolytic reactions (Stewart, C. M., Watson, A. J., Dadswell, H. E., and Kottek, J. F., unpublished work).

[1] Jacquiot, C., *C.R. Acad. Sci., Paris*, **225**, 434 (1947).
[2] Stewart, C. M., Amos, G. L., and Harvey, L. J., *Austral. J. Biol. Sci.*, **6**, 21 (1953).
[3] Hillis, W. E., *Austral. J. Biol. Sci.*, **9**, 263 (1956).
[4] Wise, L. E., "Wood Chemistry" (Reinhold Pub. Corp., New York, 1944).
[5] Freudenberg, K., *Mitt. Österr. Ges. Holzforsch.*, **1952** (3), 72.
[6] Hillis, W. E., and Carle, A., *Holzforschung*, **12**, 136 (1958).
[7] Bonner, J., "Plant Biochemistry", 129 (Academic Press, Inc., New York, 1950). Wardrop, A. B., *Tappi*, **40**, 225 (1956).
[8] Byerrum, R. U., Flokstra, J. H., Dewey, L. J., and Ball, C. H., *J. Biol. Chem.*, **210**, 633 (1954). Siegel, S. M., *Quart. Rev. Biol.*, **31**, 1 (1956). Stewart, C. M., *Tappi*, **40**, 244 (1956).
[9] Dadswell, H. E., and Hawley, L. F., *Indust. Eng. Chem.*, **21**, 973 (1929). Phillips, M., Davidson, J., and Weihe, H. D., *J. Agric. Res.*, **43**, 619 (1931).
[10] Joslyn, M. A., and Phaff, H. J., *Wallerstein Lab. Commun.*, **10**, 39 (1947).
[11] Allen, C. E., *Bot. Gaz.*, **32**, 1 (1901).
[12] Stewart, C. M., McPherson, A. J., and Frydman, L., *Aust. Pulp Paper Indust. Tech. Assoc. Proc.*, **10**, 298 (1956).
[13] Fruton, J. S., and Simmonds, S., "General Biochemistry" (John Wiley and Sons, Inc., New York, 1958).

COBALT IN NITROGEN FIXATION BY A LEGUME

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OUR understanding of cobalt as a micro-nutrient element required by animals and various micro-organisms centres around the role of vitamin B_{12}. This vitamin is required by most animal species[1] in the formation of hæmoglobin and may be concerned with protein metabolism[2]. Most workers agree that vitamin B_{12} is synthesized only by micro-organisms[1]. Many micro-organisms require preformed B_{12} (refs. 3–5); others, including *Rhizobia*[6], produce the vitamin.

Cobalt has not been demonstrated as being a requirement for the growth of higher plants, although it has been shown to be essential for blue-green algae[7]. With higher plants, cobalt supplements have been reported to increase growth of the rubber plant and tomatoes[8] and to increase elongation of pea stem sections[9,10]. Recently, Ahmend and Evans[11] have suggested that soy-bean plants grown with symbiotically fixed nitrogen as their only source of this nutrient were "distinctly benefited" from small quantities of cobalt. The experiment reported here makes it quite clear that enormous reductions in the amount of nitrogen fixed by inoculated lucerne plants result when culture solutions are sufficiently purified and controlled with respect to cobalt contaminants. Other experiments in progress at the present time at the University of California, where higher degrees of control have been obtained more recently, are giving further evidence of correspondingly greater yield differentials between inoculated lucerne plants grown on cobalt-deficient and cobalt-sufficient culture solutions.

An essential role of cobalt in symbiotic nitrogen fixation and possibly in all biological nitrogen-fixation processes is suggested by the following information. The nodules of legume plants contain hæmoglobin very similar to that of animal blood[12,13]. Nitrogen fixation is directly related to the hæmoglobin content of the nodule[14–17]. Vitamin B_{12} has been found to be concentrated in the root nodules of legume plants and particularly those that contain hæmoglobin[18]. The synthesis of hæmoglobin in the nodule is a result of the symbiotic relationship since it cannot be synthesized by either the plant or the micro-organism alone[12]. A possible relationship of cobalt to a biological nitrogen-fixing process is suggested from the results of Holm-Hansen and co-workers[7]. In their demonstration of a cobalt requirement for the growth

Table 1. Nitrogen Fixed by *Medicago sativa* (Lucerne) with and without Cobalt Supplements

Purification of salts	Cobalt supplement	Inoculation	Sodium chloride supplement	Dry wt. of plants gm./pot	Dry wt. of nodules gm./pot	Nitrogen (fixed) mgm./pot	Nitrogen (in plants) per cent
None	0	0	0	2·3	0	0	0·83
Yes	0	+	0	6·1	0·19	34	1·07
Yes	0	+	0·5 m*M*	6·6	0·20	27	0·79
Yes	0·1 μM	+	0	11·9	0·53	237	2·33
Yes	0·1 μM	+	0·5 m*M*	9·2	0·39	154	2·06
None	0	+	0	8·0	0·26	160	2·39

of blue-green algae, these workers noted that upon their cultures with low cobalt content, growth reduction of the nitrogen-fixing species was more pronounced than for the species requiring combined nitrogen.

The experiment reported here was undertaken to observe whether or not cobalt supplies could effect nodulation and symbiotic nitrogen fixation in lucerne (*Medicago sativa*). The culture solutions were restricted as to nitrogen salts and contained 1·3 m*M* nitrogen, 2·0 m*M* phosphorus, 3·1 m*M* potassium, 4·0 m*M* calcium, 1·0 m*M* magnesium, 5·0 μM manganese, 2·0 μM zinc, 0·5 μM copper, 17·5 μM iron (from Fe-ethylenediaminetetraacetic acid), 5·0 m*M* sulphur, 25 μM boron, 0·5 μM molybdenum, 22·5 μM chlorine and 1·0 gm. calcium carbonate per litre. Treatment variables are described in Table 1. All chemicals used in formulating the 'purified' cultures were treated to remove contaminant cobalt in so far as possible with the purification processes mentioned here. All water, volatile acids, bases and organic reagents used were redistilled in 'Pyrex' glass apparatus. Solutions of 2·0 *M* calcium nitrate, 1·0 *M* $K_{1\cdot1}H_{1\cdot9}PO_4$ and 0·5 *M* potassium sulphate were purified by iron-oxinate coprecipitation at *p*H 5·1–5·2 as described by Hewitt[19]. Dithizonate extraction at *p*H 8·5 was used in preparing low cobalt solution of magnesium sulphate. Iron was purified by extracting as ferric chloride with ether from hydrochloric acid solution[20]. Reagents supplying the micro-nutrients, except iron, and sodium chloride were twice recrystallized before use. The calcium carbonate added to maintain the reaction of the cultures above *p*H 6 was precipitated from purified calcium nitrate solution.

Lucerne plants were grown in a glasshouse at Berkeley during May and June. The treatments were arranged in a randomized block design and repeated four times. Three 10-day-old lucerne plants were transplanted to 2-litre, continuously aerated cultures. The following day the pots were inoculated with 1 ml. of a suspension containing a mixture of two effective *Rhizobia* strains—approximately 10^4 bacteria per pot. The plants were harvested six weeks after transplanting, separated into tops, roots and nodules, dried and weighed. Total nitrogen was determined in all plant parts.

The fixed nitrogen supplied by the culture solutions was adequate for normal growth of the plants of all treatments for four weeks. In the fifth week nitrogen deficiency symptoms developed on the plants of the 'not inoculated' treatments as well as those of the 'inoculated-minus cobalt' treatments. Nodules were visible on the roots of the inoculated plants within three weeks. No nodules were found on the plants which were not inoculated. Nodule counts made four weeks after transplanting showed the 'plus cobalt' and 'unpurified' cultures as having plants with larger nodules than the plants deprived of cobalt. The 'not inoculated' and 'minus cobalt' plants displayed characteristic symptoms of nitrogen starvation, whereas inoculated plants having cobalt supplements were green and obviously not short of nitrogen.

Whole plant weights, nodule weights and the nitrogen content of the plants are given in Table 1. Addition of cobalt to the 'purified' cultures increased plant growth by 66 per cent during an evident stress period of only two weeks. This effect of cobalt is significant at odds of 2,000 : 1. Similarly, nodule weight was doubled—significant at odds of 200 : 1.

In the presence of *Rhizobia* but without added cobalt the plants fixed 34 mgm. of nitrogen without sodium chloride and 27 mgm. of nitrogen with sodium chloride. However, with cobalt added the corresponding cultures fixed 237 and 154 mgm. of nitrogen, respectively. The variable of 0·5 m*M* sodium chloride in the culture solution which resulted in a lesser amount of nitrogen being fixed in each case was introduced into the experiment in order to give other levels of plant growth not associated with cobalt. Within this experiment, however, cobalt was by far the overriding factor in determining the amount of nitrogen fixed by inoculated *Medicago sativa*.

The magnitude of the results obtained here distinctly implies an essential role of cobalt in symbiotic nitrogen fixation. Additional results from this and an expanded confirming experiment are to be published elsewhere.

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[1] Davis, G. K., in Trace Elements Proc. Conf., Wooster, Ohio, 1957 (Academic Press, Inc., New York, 1958).
[2] Arnstein, H. R. V., and Simkin, J. L., *Nature*, **183**, 523 (1959).
[3] Starkey, R. L., *Soil Sci.*, **79**, 1 (1955).
[4] Young, R. S., *Sci. Prog.*, **44**, 16 (1956).
[5] Lockhead, A. G., and Thexton, R. H., *J. Bact.*, **63**, 219 (1952).
[6] Vyas, S. R., and Prasad, N., *J. Sci. India Res.*, **15C**, 52 (1956).
[7] Holm-Hansen, O., Gerloff, G. C., and Skoog, F., *Physiol. Plantarum*, **7**, 665 (1954).
[8] Bolle-'ones, E. W., and Mallikarjuneswara, V. R., *Nature*, **179**, 738 (1957).
[9] Thimann, K. V., *Amer. J. Bot.*, **43**, 241 (1956).
[10] Brian, P. W., and Hemming, H. G., *Naturwiss.*, **44**, 593 (1957).
[11] Ahmend S., and Evans, H. J., *Biochem. Biophys. Res. Com.*, **1**, 271 (1959).
[12] Keilin D., and Wang, Y. L., *Nature*, **155**, 227 (1945).
[13] Virtanen, A. J., and Laine, T., *Nature*, **157**, 25 (1946).
[14] Keilin, D., and Smith, J. D., *Nature*, **159**, 692 (1947).
[15] Magee, W. E., and Burris, R. H., *Plant Physiol.*, **29**, 199 (1954).
[16] Cheniae G. M., and Evans, H. J., *Biochim. Biophys. Acta*, **26**, 654 (1957).
[17] Nicholas, J. D. J., in "Nutrition of the Legumes", 239 (Academic Press, Inc., New York, 1958).
[18] Levin, A. P., Funk, H. B., and Tendler, M. D., *Science*, **120**, 784 (1954).
[19] Hewitt, E. J., Commonwealth Bur. Hort. and Plant. Crops, Tech. Com. 22 (1952).
[20] Sandell, E. B., "Colorimetric Determination of Traces of Metals", 2nd ed. (Interscience Publishers, Inc., New York, 1950).

LETTERS TO THE EDITORS

PHYSICS

The Possibility of a Self-sustaining Corbino Disk

An impressed radial current and an axial magnetic field combine in the Corbino disk[1] to induce a circulating component of current due to the Hall effect. Whichever sense the axial field may have, it is aided by the field of the circulating currents themselves, provided that the radial current is always inwards. This applies to a material with the normal sign of Hall coefficient for n-type conduction.

Without the impressed axial field, the disk would be a two-state element, which could form the basis of a digital computer. The two states would be self-sustaining current vortices of opposite spin. The existence of such vortices is suggested by a solution of the field-equations for an indefinitely thin disk of infinite radius, immersed in a medium of permeability μ:

$$J_r = \frac{I}{2\pi r.\delta z} \qquad J_\theta = \sigma.E_\theta$$

$$E_\theta = R.J_r.B_z \qquad J = \frac{\text{curl } B}{\mu.\eta_0}$$

where r,θ,z are cylindrical co-ordinates; I, total current (amp.); J, current density (amp./sq. metre); E, electric field intensity (volts/metre); R, Hall coefficient (metre^3/coulomb); σ, conductivity (mho/metre); $\eta_0 = 4\pi \times 10^{-7}$ henry/metre.

B_z, E_θ and J_θ all satisfy an equation of the form:

$$\frac{dB_z}{dr} + \mu.\eta_0.\frac{\sigma.R.I.B_z}{2\pi r.\delta_z} = 0$$

which has the solution: $B_z = A.r^{-k}$

where A is a constant of integration, and k is $\sigma\mu\eta_0 R.I/2\pi\delta z$.

More significant than the increase towards the centre in the flux density B_z is the increase in total flux within a ring of width δr when $k \geqslant 1$. This establishes a critical value for the radial current, above which amplification and regeneration are possible. A transformer exploiting the creation of a secondary flux in excess of the primary flux would behave as a magnetic power amplifier. The introduction of a magnetic feedback path in such a transformer would lead to a bi-stable trigger circuit.

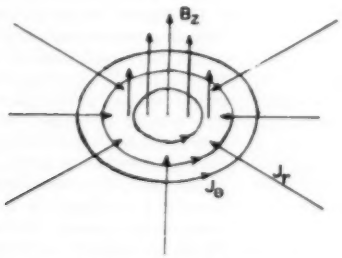

Fig. 1. Axial magnetic field in relation to the radial and circulating components of the current density

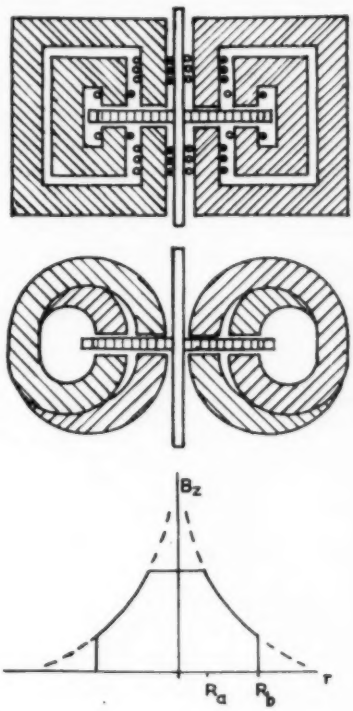

Fig. 2. Top: A magnetic amplifier having an input coil to provide B_z at R_b and an output coil at R_a. (Permeable material is diagonally shaded and Hall-sensitive material vertically shaded. The radial current is introduced by a conducting rim on the disk and is removed along the two halves of the axis)
Centre: A magnetic feedback circuit for a two-state element. (If a fraction f of B_z at R_a is returned to R_b, the fields become self-sustaining when $k = \frac{\ln(f)}{\ln(R_a/R_b)}$
Bottom: Axial flux density, circulating current density and circulating electric field-strength for an infinite disk: – – –. The same for a finite disk: ———

A vital question is whether the critical current is necessarily destructively large, as it would be for the conductivities and small Hall coefficients of ordinary metals at room temperature. However, products of conductivity and Hall coefficient of the order unity have been observed in germanium alloys at low temperatures[2]. Assuming that, by restricting the thickness of the disk to 0·01 per cent of the magnetic path, an effective permeability of 10^4 could be maintained, a current of 5 amp. per cm. of magnetic path would be required for criticality.

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[1] Von Corbino, O. M., *Phys. Z.*, **12**, 561 (1911).
[2] Debye, P. P., and Conwell, E. M., *Phys. Rev.*, **93**, 693 (1954).

Rheological Properties of Barytes Suspensions

The rheological properties of suspensions, which are used in coal preparation by dense-medium washing, are being studied. Initially, suspensions of barytes prepared by ball milling and containing particles of all sizes less than 250μ, and in most cases less than 100μ, were investigated. The effects of the concentration of solids and of the particle size of the solids on the consistency curve have been studied. It is hoped to extend the work to more complex systems containing three solid components (barytes, coal and shale) each or all of which may be deficient in smaller-sized particles. The present communication is intended to direct attention to some interesting features of the results obtained.

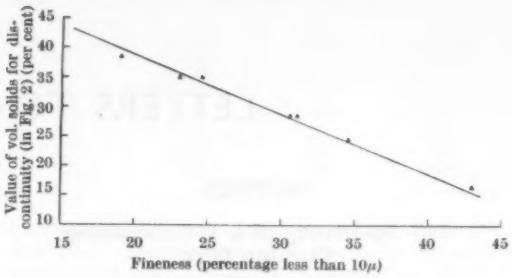

Fig. 3

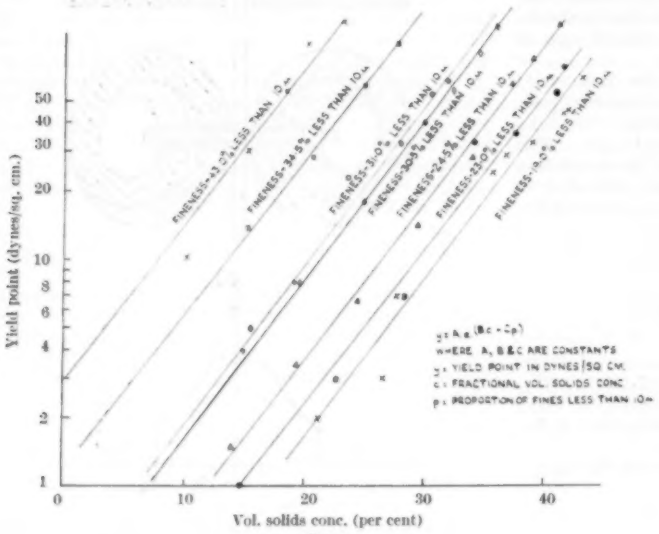
Yield point (dynes/sq. cm.)
Vol. solids conc. (per cent)
FINENESS=43·0% LESS THAN 10μ
FINENESS=34·8% LESS THAN 10μ
FINENESS=30·0% LESS THAN 10μ
FINENESS=30·6% LESS THAN 10μ
FINENESS=24·5% LESS THAN 10μ
FINENESS=23·0% LESS THAN 10μ
FINENESS=9·0% LESS THAN 10μ
$y = A\,e^{(Bc - Cp)}$
WHERE A, B & C ARE CONSTANTS
y = YIELD POINT IN DYNES/SQ. CM.
c = FRACTIONAL VOL. SOLIDS CONC.
p = PROPORTION OF FINES LESS THAN 10μ

Fig. 1

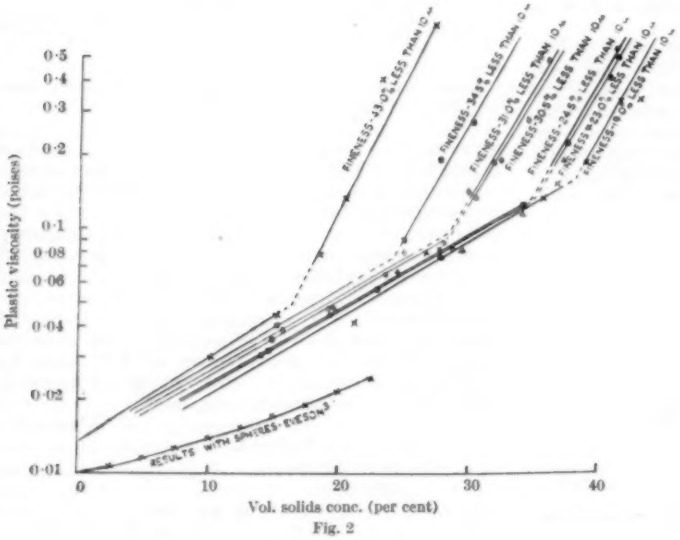

Fig. 2

The work was carried out using a Ferranti portable (rotating cylinder) viscometer[1]. This enabled the consistency to be studied over a fairly wide range of shear-rates (68–950 sec.$^{-1}$). Readings were taken immediately after thorough stirring, which could lead to the presence of a third phase (air bubbles) in the system. However, it is likely that suspensions used in practice will also contain air bubbles. All the consistency curves reveal that the suspensions are Bingham fluids over the range of shear rates used.

From the consistency curves, the yield point and plastic viscosity were estimated for suspensions containing different solids concentrations and containing solids over a range of fineness. For the suspensions studied, the results are illustrated in Figs. 1 and 2. The concentrations, at which the discontinuity in the plastic viscosity vs. concentration relationship occurs, have been found to bear a linear relation to the fineness of the barytes as expressed by the weight less than 10 microns (Fig. 3). This suggests that the phenomenon is due to the irregularities in the particles carrying immobile layers of liquid as proposed by Ward and Whitmore[2], and should enable the mean thickness of the layer to be estimated.

In earlier investigations a Gallenkamp universal torsion viscometer had been used in viscosity measurements on suspensions. A study of mode of operation and the results obtained on this instrument and the rotating cylinder viscometer lead us to conclude that, to a first approximation, the readings on the torsion viscometer (using 11/16-in. and 1$\frac{1}{4}$-in. cylinders) were a measure of the yield point of the suspensions (see Fig. 4).

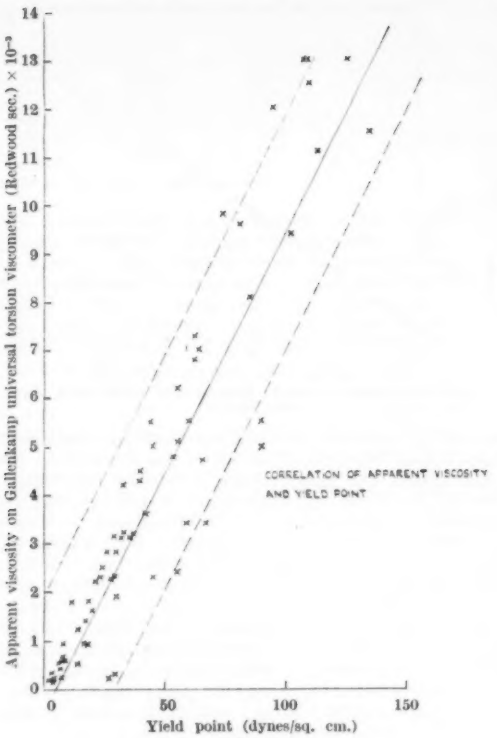

Fig. 4

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[1] Reiner, M., "Deformation Steam and Flow", 34 (Lewis, London).
[2] Ward, S. G., and Whitmore, R. L., *Brit. J. App. Phys.*, **1**, 325 (1950).
[3] Eveson, G. F., *J.O.C.C.A.* (Feb. 1959).

An Energy Band Interpolation Scheme, with Application to Body-centred Cubic Lithium

An interpolation scheme has been developed for the calculation of the energy bands in solids, using plane wave expansions of the wave functions and accurately known values of the energy-levels at high-symmetry points of the Brillouin zone. For valence electron states having the same symmetry as those of the ion cores, these expansions must be modified by the use of an additional repulsive pseudo-potential V_r. Of various suggested forms[1] of V_r, that found most useful was that of Gáspár[2] and Antončík[3], an extension to metals of that derived for atoms by Gombás[4]. By using only a small number of terms in the expansions it was then found possible to reproduce the energy-levels at high-symmetry points with at least the accuracy to which they had been originally determined. The levels at a large number of general points of the zone were then calculated, and, in order to obtain the density of states $N(E)$, these energy values were fitted by a symmetrized triple Fourier expansion of $E(k_x,k_y,k_z)$.

The method has been applied to body-centred cubic lithium, for which energy-levels at high-symmetry points have been obtained by Glasser and Callaway[5,6], using the orthogonalized plane wave method and the corrected Seitz potential[7], and with a stated accuracy $\pm 0{\cdot}05$ Ry. The number of terms used in the plane wave expansions to reproduce these levels was taken to be equal to the degeneracy of the corresponding level in the free-electron approximation (for example, 6 for the lowest state H_1, in the notation of Bouckaert *et al.*[8]). For the s-like levels V_r involves the $1s$ wave function of the ion core, which was chosen to be hydrogenic with a screening constant which was taken to be a disposable parameter, fixed by giving agreement with the lowest state Γ_1. To reproduce the p-like levels, the truncation of the plane wave expansions was brought about by using as a correction a very small further pseudo-potential, while the d-like levels could be reproduced by using the Seitz potential alone. The lower levels thus calculated agreed with those of Glasser and Callaway to within their stated accuracy. Levels were then calculated at general points within the first zone on a lattice $(a/2\pi)(k_x,k_y,k_z) = (1/10)n$ $(n = 1,2\ldots)$ by using an appropriate number of terms of the plane wave expansion, and the first 22 Fourier coefficients of $E(k_x,k_y,k_z)$ obtained by least squares. Finally, $N(E)$ was obtained by numerical integration. The calculations were carried out on the Mercury Computer of the University of London.

Fig. 1 shows the calculated $N(E)$ curve, with E_F, the Fermi energy, $-0{\cdot}425$ Ry., and E_N, the lower energy at N, $-0{\cdot}400$ Ry., N being the point on the surface of the first zone nearest to its centre Γ. Since $E_F < E_N$, the calculation thus shows that the Fermi surface does not touch the zone boundary; however, $(E_N - E_F)$ is only equal to $0{\cdot}025$ Ry., which is within the accuracy of Glasser and Callaway. The broken curve on Fig. 1 gives the $N(E)$ curve

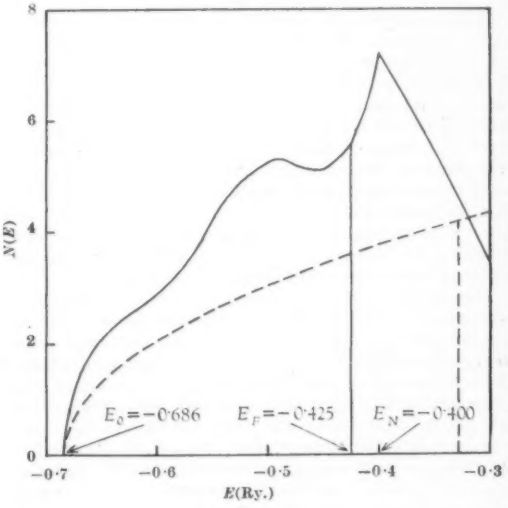

Fig. 1

for free electrons. The energy surfaces near Γ can be expressed as[7]:

$$E(\mathbf{k}) = E_0 + E_2k^2 + E_4^{(1)}k^4 + 5E_4^{(2)}(k_x^2k_y^2 + k_y^2k_z^2 + k_z^2k_x^2 - 1/5k^4)$$

where $E_0 = -0{\cdot}686$, $E_2 = 0{\cdot}754$, $E_4^{(1)} = -0{\cdot}064$, $E_4^{(2)} = -0{\cdot}146$, and where E_2 corresponds to an effective mass at Γ, $m^* = 1{\cdot}32\ m_e$, and $E_4^{(2)}$ gives a measure of the anisotropy. Previous values of these constants are given in refs. 6 and 9. The value $E_F - E_0 = 0{\cdot}261$ Ry. compares with a value 0·235 Ry. based on the soft X-ray emission spectrum[10]. The calculated low-temperature electronic heat coefficient is $2{\cdot}38 \times 10^{-4}$ cal. mol.$^{-1}$ deg.$^{-2}$, compared with the observed value[11] $4{\cdot}18 \times 10^{-4}$, which was, however, not obtained for body-centred cubic lithium.

The method is now being applied to other metals including sodium, potassium, titanium and beryllium, and fuller details will be published elsewhere.

We are indebted to Prof. H. Jones for helpful discussions, and one of us (J. F. C.) acknowledges the award of a University Postgraduate Studentship of the University of London.

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[1] Phillips, J. C., and Kleinman, L., *Phys. Rev.*, **116**, 287 (1959).
[2] Gáspár, R., *Acta Phys. Hung.*, **9**, 79 (1958).
[3] Antončik, E., *J. Phys. Chem. Solids*, **10**, 314 (1959).
[4] Gombás, P., "Handbuch der Physik", **36**, 109 (1956).
[5] Glasser, M. L., and Callaway, J., *Phys. Rev.*, **109**, 1541 (1958).
[6] Callaway, J., *Solid State Physics*, **7**, 99 (1958).
[7] Kohn, W., and Rostoker, N., *Phys. Rev.*, **94**, 1111 (1954).
[8] Bouckaert, L. P., Smoluchowski, R., and Wigner, E. P., *Phys. Rev.*, **50**, 58 (1936).
[9] Cohen, M. H., and Heine, V., "Adv. in Physics", **7**, 395 (1958).
[10] Bedo, D. E., and Tomboulian, D. H., *Phys. Rev.*, **109**, 35 (1958).
[11] Roberts, L. M., *Proc. Phys. Soc.*, B, **70**, 744 (1957).

Trace Analysis by X-Ray Fluorescence using Ion Exchange Resins

The analysis of trace elements in concentrations of a few parts per million, or less, by X-ray fluorescence spectrography has hitherto not been practicable, mainly as a result of the relative weakness of the emitted spectra. Grubb and Zemany[1] have obviated this difficulty by showing that traces of cobalt at levels of 1 μgm./l. may be analysed by first adsorbing the element on to a small piece of cation membrane and irradiating this in an X-ray spectrograph. The long equilibration times involved (16–48 hr.) have, however, precluded the general application of their method in a wider field, particularly in process control, where the time-factor is important.

The method which we have developed is based on a similar concentration step, but the element to be determined is collected on an ion-exchange resin (granular or liquid), after which the resin is irradiated directly in the X-ray spectrograph. A number of elements may thus be collected and determined simultaneously without the necessity of time-consuming separations. We have found that rapid equilibrium with solid resins is attainable, provided that the resin is soaked beforehand. Equilibrium with liquid resins is even faster. The relative amounts

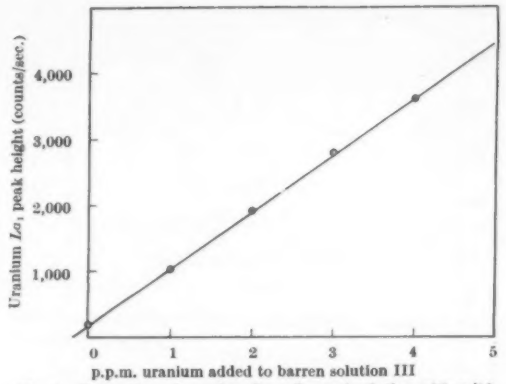

Fig. 1. Extraction of uranium from 'barren' solution with solid resin, '*AG*–1*X*'

of resin and solution are so chosen that the enrichment ratio brings the concentration of the element on the resin within the range where it can be determined directly by X-ray spectrography. Provided that the variation in particle size of the solid resin is small, no extra variance is introduced in the results by distributional variations in the resin bed. Where relatively large amounts of co-adsorbed elements give rise to interferences, such as absorption or enhancement of the measured radiation, the effects can be overcome by the use of suitable internal standards. The voids between the solid resin particles are filled with the reference element in non-adsorbing form. The reproducibility of the results obtained justify the introduction of standards in this way.

The determination of uranium in barren sulphate effluent, from the ion-exchange columns in the Witwatersrand uranium industry, serves as a good illustration of this technique. (Detailed results will be published elsewhere.) The major ionic constituents of these solutions are:

Fe^{2+} (2–3 gm./l.) Fe^{3+} (2–3 gm./l.)
Mn^{2+} (4–6 gm./l.) Al^{3+} (4–6 gm./l.) and
SO_4^{2-} (~20 gm./l.) at a *p*H of about 3

The uranium content varies between 0 and 3 p.p.m. 500 ml. of this solution is shaken for 5 min. with 2 gm. (dry weight) of '*AG*–1*X*' anionic resin in sulphate form which has been soaked beforehand in 0·25 *M* ammonium sulphate solution. The resin is filtered by

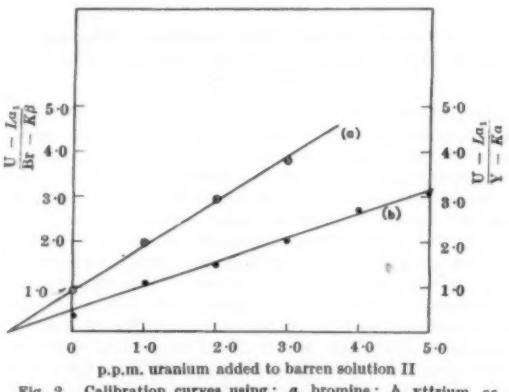

Fig. 2. Calibration curves using: *a*, bromine; *b*, yttrium, as internal standards with the '*AG*–1*X*' extractions

Table 1. Sample Uranium Determinations in Barren Solutions

'AG–1X' resin		20 per cent 'LA–2' in kerosene	
Uranium added (p.p.m.)	Uranium found (p.p.m.)	Uranium added (p.p.m.)	Uranium found (p.p.m.)
1·70	1·68	2·30	2·44
3·76	3·60	3·40	3·50
4·35	4·30		

suction, washed over into a suitable liquid sample holder[2] and irradiated (Philips high-intensity molybdenum tube). The linearity obtained when making standard additions of uranium to barren solutions is illustrated in Fig. 1. Iron, probably co-adsorbed as negative ferric sulphate complexes, absorbs the uranium-$L\alpha_1$ radiation, so that the use of internal standards is advisable where the iron content of the solutions tends to fluctuate. (In practice, we have found this unnecessary as the iron concentration in these barren solutions remains constant within narrow limits.) Fig. 2 illustrates the feasibility of using alcoholic bromobenzene and aqueous yttrium sulphate as internal standards. Under the above conditions about 90 per cent of the uranium in a solution containing 2 p.p.m. is extracted with good reproducibility (standard deviation 1–2 per cent).

Good extraction is also possible with anionic liquid resins (for example, 'Amberlite *LA*–2'). The sensitivity is somewhat lower (see Figs. 1, 3), as the highest practicable concentration ratio is only about 50. The linearity obtainable when extracting 500 ml. of 'barren' solution with 10 ml. 20 per cent '*LA*–2' in kerosene is shown in Fig. 3. Although much less iron is co-extracted in this case, so that absorption effects are not serious, we have established that internal standard procedures can also be applied here by using bromobenzene in kerosene. Typical results are given in Table 1. The accuracy of both variants of the method is estimated at 5 per cent on 1 p.p.m. uranium, the main source of error residing in the statistical counting error due to the unfavourable peak to background ratio. The limits of detection, under the above conditions, are estimated to be 0·1 p.p.m. uranium using solid resin, and 0·2 p.p.m. using liquid resin.

As the method is also applicable to cationic resins, we believe that this technique extends the scope of X-ray fluorescent analysis far beyond its present limits, and its rapidity should render it useful for the routine trace analysis of a wide range of elements.

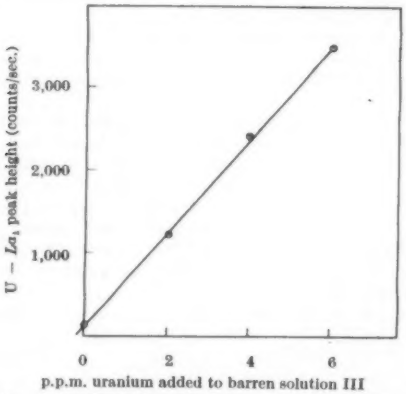

Fig. 3. Extraction of uranium from 'barren' solution using liquid resin, 'Amberlite *LA*–2'

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[1] Grubb, W. T., and Zemany, P. D., *Nature*, **176**, 221 (1955).
[2] Van Niekerk, J. N., and Wybenga, F. T., *J. App. Spectr.* (in the press).

Micromosaic Patterns in Single Crystals of Potassium Chloride containing Barium Chloride

Dislocation networks generated in alkali halide crystals when subjected to mechanical strain have been rendered visible by 'decoration' with colloidal aggregations of colour centres[1] and with silver[2]. Amelinckx *et al.*[3] obtained micromosaic patterns in sodium chloride crystals containing sodium or barium chloride and Hedges and Mitchell[4] in silver bromide containing excess silver.

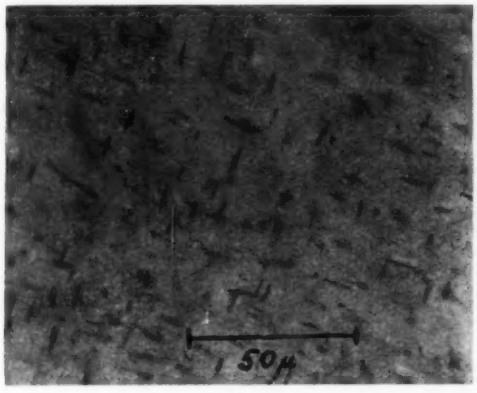

Fig. 1

In the present work barium chloride in concentrations varying from 5×10^{-5} to 10^{-3} barium/potassium atoms was added to melts of potassium chloride and single crystals grown from the melt by the Kyropoulos method[5]. The crystals were cleaved to produce specimens of about 0·5 mm. thick and microphotographs made of interior planes at a magnification of 500. It was found that crystals containing about 5×10^{-4} barium/potassium showed throughout the interior a pattern consisting of a network of lines some 5–10μ long (Fig. 1). Pure crystals showed no such patterns. Occasionally, deep within the crystal, was seen a pattern such as that shown in Fig. 2, which gave the impression of voids within the crystal some 5μ or less in depth and some hundreds of microns in length. It might be supposed that such voids might be generated as a means of reducing the strains set up by the relatively high impurity content or by the unavoidable thermal strains in the formation of the crystals. It has not been possible so far to anneal out such voids even after many hours heating and of subsequent cooling to room temperature at a rate of about 5 deg. C. per

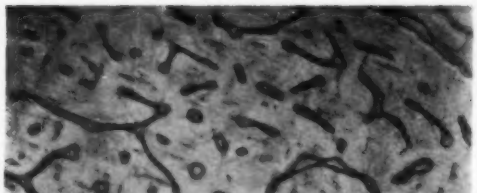

Fig. 2

hr. The pattern of fine lines seen in Fig. 1 might be interpreted in two ways. It might be the result of dislocations produced by the added barium chloride, a minimum concentration being necessary and a too high concentration resulting in such extensive disorganization of the lattice that no simple pattern is formed or can be discerned. Or, the pattern may be already present in the 'pure' crystal in the form of invisible internal surfaces or lines and is revealed by precipitation on these surfaces or lines of an added substance such as barium chloride when this is present within certain limits of concentration. High concentration of barium chloride produces a cloudiness which can be cleared again by heating. It is not possible from the present observations to decide between the two possibilities, but it seems that the incorporation of a suitable impurity in single crystals of alkali halides reveals a defect structure which might be used in the study of the formation and behaviour of such structures and the part they play in the behaviour of crystals under various conditions.

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[1] Hosoya, S., *J. Phys. Soc. Japan*, **11**, No. 12, 1231 (1956).
[2] van der Vorst, W., and Dekeyser, W., *Phil. Mag.*, **1**, No. 9, 882 (1956).
[3] Amelinckx, S., van der Vorst, W., Gevers, R., and Dekeyser, W., *Phil. Mag.*, **46**, 450 (1955).
[4] Hedges, J. M., and Mitchell, J. W., *Phil. Mag.*, **44**, 223 (1953).
[5] Kyropoulos, S., *Z. anorg. allgem. Chemie*, **154**, 308 (1926).

RADIATION CHEMISTRY

Polymer Formation in Irradiated *p*-Xylene

Investigations of the radiochemical behaviour of alkylbenzenes have predominantly been focused on the gaseous decomposition products, and data about the liquid reaction products are rather scarce.

As part of a study of the kinetics of radiolysis of alkylbenzene mixtures, *p*-xylene was submitted to mixed atomic pile radiation.

In view of the complex nature of mixed atomic pile radiation, a direct determination of the amount of energy absorbed by the sample is not always possible. For the work reported here it was chosen to use biphenyl as a reference component.

Biphenyl has been extensively studied in relation to its use as a moderator, and by choosing it as an irradiation standard it is expected to make possible a direct comparison of the data obtained on the lighter alkylbenzenes with the data which have already been accumulated for heavier products such as the terphenyls.

In addition to samples of pure *p*-xylene and biphenyl a certain number of mixtures of *p*-xylene and biphenyl were irradiated and analysed. The *p*-xylene and biphenyl both had a purity of 99 + per cent.

4-gm. samples of *p*-xylene, biphenyl or a mixture of both were sealed under vacuum in silica capsules (diam. 2 cm., height 6 cm.). Each capsule was placed in a tightly fitting aluminium container and irradiated in the *Y*7 channel of the *BR* I reactor at Mol, Belgium. The equilibrium temperature of the samples during irradiation was approximately 95° C.

After irradiation the liquid phase was analysed by gas-liquid partition chromatography using a 5-m. stainless steel column (1/4 in.) packed with 30/50 mesh 'Sil-O-Cel', impregnated with 30 per cent 'D–C' high-vacuum silicone grease. Helium at a rate of 100 ml./min. was used as a carrier gas and column temperature was stabilized at 150° C. The retention time of biphenyl was about 1 hr.

For quantitative analysis of the liquid-phase naphthalene was used as a tracer, and benzene as a solvent. The area correction factors were determined with solutions of known composition at the different concentration-levels encountered.

From the measured ratio of *p*-xylene or biphenyl to naphthalene and the known ratio of naphthalene to total sample, one derives the concentration of *p*-xylene or biphenyl in the sample. In order to investigate further the nature of the polymer formed, a fraction of the irradiated sample was distilled for 1 hr. at 150° C. under a pressure of 0·05 mm. mercury, yielding a colourless distillate and a gummy brownish residue of heavy polymer tar.

The concentration of *p*-xylene or biphenyl in the distillate was determined by the same analytical technique.

From the concentration of *p*-xylene in the sample before irradiation, after irradiation, and in the distillate one calculates the amount of *p*-xylene which has disappeared by radiolysis, as well as the amount of polymer formed, which can be differentiated in compounds of the boiling range of terphenyl and heavy polymer tar.

No correction was made to account for the gaseous decomposition products, as it was found that their weight was well below 1 per cent of the total sample weight.

Furthermore, a preliminary analysis showed that the amount of liquid products of lower molecular weight formed upon irradiation was so small that it has also been neglected in the material balance calculations.

The results have therefore been treated as if the disappearance of the initial compound measures the formation of products of higher molecular weight.

Typical experimental results on *p*-xylene and biphenyl are summarized in Table 1.

Table 1. Total Incident Dosage: $1{\cdot}21 \times 10^{19}$ NVt (Thermal)

Weight (per cent)	*p*-Xylene	Biphenyl
Unchanged after irradiation	92	91
Total polymer	8	9
Heavy tar	2	7

From the results obtained at an incident dosage of $1{\cdot}21 \times 10^{18}$ nVt (thermal) (Table 1) and those obtained at $0{\cdot}76 \times 10^{18}$ nVt (thermal) it follows that *p*-xylene polymerizes according to the same rate law as biphenyl, which means that the reaction is a first-order process[1].

Mixtures of *p*-xylene and biphenyl varying in composition from 4·75 to 28·5 per cent weight of *p*-xylene were irradiated under the same conditions. The experimental results show that within the limit of accuracy of the analytical method the first-order rate law also applies. The results for heavy tar formation are not so conclusive as those concerning the *p*-xylene/biphenyl ratio. The rather large scatter of the results about heavy tar content versus *p*-xylene/biphenyl ratio may be attributed to the limitations of the analytical method used. The general trend of the results is, however, in agreement with a first-order rate law.

From the similarity of behaviour of *p*-xylene and biphenyl under the influence of mixed pile radiation one may also expect a similar behaviour with respect to gamma irradiation. As regards the radiochemical polymerization yield $G_p = \frac{\text{molecules polymerized}}{100\ \text{eV.}}$ of *p*-xylene, its magnitude may be taken as approximately 1·45 times the corresponding value for biphenyl.

A more detailed account will be published elsewhere.

I am indebted to Dr. F. Dupire for his advice about the chromatographic technique and to M. A. de Wilde for his interest in this subject.

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Use of Ionizing Radiation in Spot Tests for Nitrogenous Compounds

SEVERAL spot tests are available for determining substances containing nitrogen, and are widely used in qualitative and semi-quantitative analysis[1]. All these involve the development of colour or fluorescence by spraying with suitable reagents. We have discovered that many nitrogenous compounds give a brown colour when spotted on to filter paper and exposed to doses of ionizing radiation in the range 5×10^6–2×10^8 rads.

The results described below stem from an observation by one of us that ammonium dihydrogen phosphate spotted on filter paper and exposed to a flux of 10^{12} neutrons/cm.²/sec. in the reactor *Bepo* for six days developed a brown colour. In subsequent work we used a 4-MeV. linear accelerator as a radiation source, since this could be used to deliver 10^8 rads in a much shorter time than the reactor and without inducing any radioactivity. Several different types of filter paper were tested and Whatman No. 4 selected as the most suitable: all papers became brittle and pale yellow after 10^8 rads, and cellulose acetate papers crumbled completely away after this treatment[2]. Colours developed slowly at room temperature and faster on warming. The best conditions for colour development were obtained using aliquots containing about 20 μgm. nitrogen exposed to 10^8 rads, when the limit of detection was approximately 2 μgm. nitrogen. Under these conditions well-defined brown spots were obtained from the following materials: ammonium chloride, methylamine hydrochloride, diethylamine hydrochloride, glycine, alanine, asparagine, proline, potassium nitrate, sodium nitrite and nitrobenzene. Fainter spots were obtained with acetamide, diphenylamine, pyridine and azobenzene, but no spots could be found when tetramethyl ammonium bromide and cetyl trimethyl ammonium bromide were tested. β-indolyl acetic acid gave a pink spot, while aspartic acid and trimethylamine hydrochloride were anomalous in giving deep brown spots on heating (with or without ionizing radiation).

The colour formation is probably due to a Maillaird reaction between amino-groups and aldehydic substances resulting from radiation-induced breakdown of cellulose. Presumably nitro groups are reduced to amino groups under these conditions of irradiation, and tertiary amines like pyridine undergo ring cleavage. However, no improvement in colour development could be obtained by soaking the filter paper in glucose solution before spotting and irradiating. As an analytical technique, the reaction is not so sensitive as the ninhydrin reaction for amino-acids, which can detect about 0·1 μgm. nitrogen, but it might be useful for detecting other substances which do not give the ninhydrin test. It has been used to develop paper chromatograms of amino-acids.

Finally, the reaction may be of interest to those concerned with radiation dosimetry. The fact that a colour will develop at 5×10^6 rads, which is near the sterilizing dose for food and pharmaceutical materials, is important in this connexion.

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[1] Feigl, F., "Spot Tests in Organic Analysis" (Elsevier, 1956).
[2] Saeman, J. F., Millett, M. A., and Lawton, E. J., *Indust. Eng. Chem.*, **44**, 2848 (1954).

Radiation Synthesis of Urea

LOISELEUR and PETIT[1] have reported that urea is formed in the X-irradiation of an ammoniacal and methanolic solution of *m*-phenylenediamine and dichloromethane in the presence of air. A further report has been given recently by Loiseleur, Petit and Clémentin[2] on the effect of oxygen-acceptors on the yield of urea. We set out to synthesize substituted ureas and to study the proposed mechanisms, but we found that no urea was formed in experiments with similar solutions.

γ-radiation from a cæsium-137 source (dose-rate: 10^{16} eV./ml./min.), taking $G_{Fe^{3+}} = 15{\cdot}5$, and X-rays from a copper target at 40 kV. and 20 m.amp. (dose rate: $1{\cdot}5 \times 10^{15}$ eV./ml./min.) were used in the experiments. Closed glass irradiation cells of 25 ml. capacity were fitted with polythene capillary tubing through which air was bubbled, after it had first passed through a vessel containing the solution to be irradiated. The solution (32 ml. methanol, 4 ml. dichloromethane, 250 mgm. *m*-phenylenediamine,

0·25 ml. 30 per cent aq. ammonia) was freshly prepared for each experiment. Reagents were carefully purified: methanol and dichloromethane were fractionated; ammonia (sp. grav. = 0·880) was diluted with distilled water; *m*-phenylenediamine was twice distilled under nitrogen and used immediately after the second distillation as well-defined light-yellow crystals (m.p. 61° C.) and xanthydrol was synthesized and used as a 10 per cent solution in ethanol[3]. After receiving total absorbed doses of γ-radiation ranging from 6×10^{17} eV./ml. to 7×10^{18} eV./ml. and of X-rays at $4{\cdot}5 \times 10^{16}$ eV./ml., the solutions were analysed for urea using Fosse's method with the xanthydrol reagent. This is the method used by Loiseleur and it might be noted that it is not specific for urea although a well-defined dixanthyl urea compound is formed[4]. (The reaction was erratic and even failed to proceed with quite large amounts of added urea when the reagent was used in the normal way.)

In all experiments where the xanthydrol reaction was used, whether the solutions were irradiated or not, well-defined white crystals were obtained in reasonable yield. Infra-red absorption spectra and elemental analysis showed quite clearly that the compound was not dixanthylurea. The empirical formula was $C_{9\cdot7}H_{4\cdot2}N_{0\cdot26}O_{0\cdot61}$.

A paper chromatographic method was therefore developed to detect urea. Various methods are available for the detection of as little as 10γ of urea[5]. However, the uni-dimensional technique with numerous solvents failed to distinguish urea satisfactorily from the more reactive *m*-phenylenediamine. The two-dimensional technique with butanol/ethanol/water (4 : 1 : 1) was adequate for more than 50γ urea. Aqueous pyridine (4 : 1) was found to allow good separations using the uni-dimensional ascending technique. The chromogenic reagent was 1 per cent dimethylaminobenzaldehyde in ethanol containing 1 ml. concentrated hydrochloric acid. After spraying, the chromatogram was heated at 100° C. for 5 min. to increase the intensity of the yellow urea spot. The limiting sensitivity of the method was 10–20 μgm. of urea in 1,400 μgm. of *m*-phenylenediamine.

Analyses by paper chromatography of aerated solutions, which were irradiated as described above, showed no trace of urea. For comparison, the lowest urea yield reported by Loiseleur and Petit (350 mgm. urea derivative per 100 ml. after an estimated $0{\cdot}75 \times 10^6$ r. of X-rays) is well within the limits of detection by chromatography. Our conclusion from these preliminary experiments, which were arranged to reproduce the conditions reported as closely as possible, is that no urea is synthesized by γ- and X-radiation of the 'Loiseleur' solution, or that the synthesis is extremely sensitive to dose-rate.

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[1] Loiseleur, J., and Petit, M., *C.R. Acad. Sci., Paris*, **240**, 1026 (1955). Loiseleur, J., *J. Chim. Phys.*, **52**, 626 (1955).
[2] Loiseleur, J., Petit, M., and Clémentin, A., *C.R. Acad. Sci., Paris*, **248**, 1720 (1959).
[3] Kny-Jones, F. G., and Ward, A. M., *Analyst*, **54**, 574 (1929). Gilman, H., and Blatt, A. H., "Organic Syntheses", Collective Vol. 1 (Wiley, New York, 1941).
[4] Elderfield, R. C., edit. by, "Heterocyclic Compounds", **2** (Wiley, New York, 1951).
[5] Milks, J. E., and Janes, R. N., *Anal. Chem.*, **28**, 846 (1956).

CHEMISTRY

Overturning of Stearic Acid Molecules in Monolayers

I have recently reported some experiments on the exchange of stearic acid molecules and potassium ions between mica surfaces brought into contact and emphasized the importance of these phenomena in lubrication[1]. I wish now to describe some further results of these experiments, and discuss their significance in terms of the motions which long-chain polar molecules in mono-molecular layers on solid surfaces may undergo.

Rideal and Tadayon[2] first reported radiometric observations on the transfer of stearic acid–carbon-14 from monolayers deposited on solid surfaces to other solids brought into contact with them. Their experiments were carried out with metal-coated mica sheets as substrates, and no measurements of the area of contact were made, although it was suggested that it was only a small fraction of the total area of the sheets. After long periods (20–100 hr.) of contact, an apparent equilibrium in the amount transferred was reached. In Table 1, their results are summarized in terms of the surface from which the stearic acid was removed.

Table 1. 'Equilibrium' Transfer of Stearic Acid; 36° C. (Rideal and Tadayon)

From	To	Amount (per cent)	From	To	Amount (per cent)
Mica	Mica	50–60	Copper	Copper	4
	Tin	85		Silver	4
	Platinum	90		Tin	6
Tin	Tin	50		Platinum	8
	Nickel	65	Nickel	Nickel	6
	Copper	85		Tin	8
	Silver	30		Copper	10
Silver	Silver	45	Platinum	Silver	15
	Copper	80		Copper	8
	Tin	65			
	Platinum	90			

As shown in Table 1, the substrates used fall into two groups: those which can give up a major fraction of the stearic acid (mica, tin, silver) are relatively inert to reaction with the fatty acid, while those which lose only a few per cent of the acid initially present (copper, nickel) are reactive metals. (Platinum seems to be somewhat anomalous; as Young[3] has suggested, this may reflect a strong physical interaction due to the thinness or absence of an oxide film on platinum.)

In the previous report[1], it was shown that the extent of transfer of a stearic acid monolayer between two mica sheets brought into contact for even a few minutes was about half that present in the area of contact. It was also found that potassium ions from the exchangeable-ion sites on the mica surfaces were exchanged, even in the presence of the stearic acid monolayer. Two other cases have now been examined qualitatively. Transfer between two monolayers of stearic acid has been observed, using one radioactive and another non-radioactive stearic acid film, both deposited on mica. Some observations of transfer from barium stearate monolayers (stearic acid spread on solution 10^{-4} *M* barium chloride and 10^{-3} *M* in potassium bicarbonate, adjusted to *p*H 9 with potassium hydroxide) on mica have also been made. In the present measurements, the technique of Rideal and Tadayon was used. The possibility of vapour-phase transport in these systems was also examined. Young[3] had observed such transport at elevated

Table 2. Observed Count Rates (per cm.²) on Mica Sheets in Contact and Transfer Experiments (each 5–10 min.) (Corrected for background count)

	Radioactive sheet Initial	Radioactive sheet Final	Inactive sheet	
Stearic acid monolayers :				
Separated 1 mm., air	650 c./m.	650 c./m.	0 c./m.	
Separated 1 mm., $p = 50\mu$	650	450	75–150	
Contact	650	—	uncoated stearic acid monolayer	18–40
				5–15
Barium stearate monolayers :				
Separated 1 mm., $p = 50\mu$	650 c./m.	620 c./m.	—	
Contact	650	—	42 c./m. (uncoated)	

temperatures, and suggested that it might account for the apparent diffusion described by Rideal and Tadayon. The present experiments were carried out at room temperature ($25 \pm 2°$ C.).

The experimental results are shown in Table 2, where count-rates observed with an end-window Geiger–Müller counter placed over the sample after the indicated exposure are listed. For the measurement of initial rates, and for the samples exposed without contact, a mask was used and an area of 1 cm.² near the centre of the sheet was examined. The samples in contact, as might be expected, showed wide variations in activity over their surfaces. The values given for them represent the count-rate of the whole sheet (observed without a mask) divided by the sheet area to give the average count-rate for 1 cm.².

As indicated in Table 2, exposure of two sheets of mica 1 mm. apart in room air shows no significant transfer after 5–10 min. After 1 hr. of such exposure, the result is the same. However, when the samples are placed in a vacuum chamber and the pressure is reduced to 50μ for 5 min., almost one-third of the activity of the radio-stearic acid monolayer is lost, and a comparable amount is found on the initially inactive sheet. With barium stearate, however, the loss of activity is much less. In contact experiments, however, no such difference appears; transfer of barium stearate and stearic acid to fresh mica surfaces is of the same order of magnitude. When a stearic acid monolayer is confronted not with a clean mica surface, but with one which is coated with another stearic acid monolayer, transfer (exchange ?) also occurs. It seems that the amount transferred is less in the latter case, although the possible variation in true contact area in these experiments prevents any definite statement on this point.

The results are in good agreement with the observations of Young on stearic acid and stearate monolayers, and support his suggestion of vapour-phase transport in the work of Rideal and Tadayon. If we consider all these results in the framework of Langmuir's mechanism for the overturning of stearate molecules in monolayers[4], correlations are obvious. The molecules in the monolayer are held in their 'normal' (polar head toward the substrate) position if there is a strong interaction between the polar head and the underlying material. This can be a chemical bond or perhaps a strong physical interaction such as that between stearates and platinum. This accounts for the strong attachment, lack of reduction of contact angle in the presence of water and lack of vapour-phase transfer with monolayers on reactive solids.

If a stearic acid monolayer is deposited on an unreactive substrate, some of the molecules may at any time be overturned. Whenever there is a possibility of anchoring these molecules in the overturned position (another surface brought into contact or a liquid water drop placed on the surface), such a process will proceed to an equilibrium determined by the minimization of the energy of the system. Analogous behaviour will be found with soap monolayers. It now appears probable that the transfer of potassium ions observed in my previous work[1] is to be associated with soap formation.

Differences should, however, be expected in the vaporization behaviour of divalent soap and free-acid monolayers on unreactive solids. The energy required to separate a barium stearate molecule from its neighbours will obviously be greater than that required to remove a single stearic acid molecule, on the basis of chain–chain interactions. This is in agreement with the experimental results.

From the point of view of lubrication phenomena, of course, it is necessary to consider liquid systems and adsorbed polar films. Studies of how the presence of bulk liquid will affect the processes described above are now in progress.

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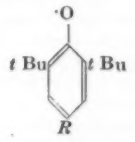
[1] Gaines, jun., G. L., *Nature*, **183**, 1110 (1959).
[2] Rideal, E., and Tadayon, J., *Proc. Roy. Soc.*, A, **225**, 346 (1954).
[3] Young, J. E., *Aust. J. Chem.*, **8**, 173 (1955).
[4] Langmuir, I., *Science*, **87**, 493 (1938).

Study of the Oxidation of Hindered Phenols by Electron Spin Resonance

The detection by electron spin resonance of the 2,6,-di-*t*-butyl-4-methylphenoxy radical during the oxidation of the corresponding phenol with lead dioxide has been reported recently[1]. The purpose of this communication is to report a similar investigation of the free radicals formed during the oxidation of solutions of the hindered phenols 2,6,-di-*t*-butyl-4-*R*-phenol [where $R \equiv$ methyl (I); ethyl (II); and *t*-butyl (III)] with an alkaline solution of potassium ferricyanide.

This method of oxidation has the advantage that only the phenoxy radicals are formed, whereas in the former case secondary radicals were soon observed. Furthermore, the hydrocarbon solution of the radical can be rapidly separated from the oxidizing medium, which permits the kinetics of radical decay to be determined.

Both the methyl and ethyl phenols, (I) and (II), give transient electron spin resonance spectra which are attributed to the corresponding phenoxy radicals, (IV) and (V). The *t*-butyl phenol (III) gives the spectrum due to the stable phenoxy radical (VI)[2].



R≡ methyl (IV)
ethyl (V)
t-butyl (VI)

The spectrum from the methyl phenoxy (IV) consists of four triplets. This is identical with the spectrum already reported[1]. The spectrum of the ethylphenoxy (V) consists of three triplets, and is attributed to the interaction of the unpaired electron with the two —CH_2— protons of the ethyl group (major splitting) together with a much smaller interaction with the two ring protons. The interaction

Table 1. Hyperfine Splitting Constants of 2,6-Di-*t*-butyl-4-*R*-phenoxy Radicals

Para-substituent *R*	Hyperfine splitting (œrsted)	
	Para-alkyl protons	Ring protons
Methyl	11·0 ± 0·1	1·65 ± 0·05
Ethyl —CH_2—	8·65 ± 0·1	1·70 ± 0·05
Ethyl —CH_3	not resolved	
t-Butyl	not resolved	1·84 ± 0·05

with the —CH_3 protons of the ethyl group is too small to give a resolvable splitting of the spectrum. This is in line with the fact that the protons of the para *t*-butyl group of the butyl-phenoxy (VI) do not give a resolvable splitting. The hyperfine splitting constants of the electron spin resonance spectra are summarized in Table 1.

The assignment of the electron spin resonance spectra to the phenoxy radicals is confirmed by the fact that all three spectra can be interpreted logically on the same basis.

The oxidation of the methyl phenol (I) yields appreciable amounts of the following dimers: 1,2-*bis*(3,5-di-*t*-butyl-4-hydroxyphenyl) ethane and 3,5,3′,5′-tetra-*t*-butylstilbene-4,4′-quinone, which suggests that the initial oxidation occurs on the methyl group to give the 4-hydroxy-3,5,-di-*t*-butylbenzyl radical. However, Cook *et al.*[3] have suggested that the following sequence of reactions occurs during the oxidation:

$$\text{phenol} \xrightarrow[[O]]{} \text{phenoxy} \xrightarrow[\text{[rearrangement]}]{} \text{benzyl} \xrightarrow[\text{[dimerization]}]{} \text{end products} \quad (1)$$

Becconsall *et al.*[1] have confirmed that the phenoxy radical (IV) is formed during the oxidation, but suggest that the next step is the formation of a complex between two phenoxy radicals, rather than a re-arrangement to the benzyl radical. There is also the possibility that the phenoxy radical decays by the reaction:

$$\text{phenoxy} + \text{phenol} \longrightarrow \text{phenol} + \text{benzyl} \quad (2)$$

The present results show that the methyl phenoxy (IV) decays with first-order kinetics, and that the rate of decay is independent of the concentration (0·1–5 per cent) of the parent phenol (I) initially present. However, the rate of decay is influenced by the solvent, and is slower in a highly polar solvent; for example, the rate constants in benzene, methyl*cyclo*hexane and acetone at 25° C. are $1{\cdot}33 \times 10^{-2}$ sec.$^{-1}$, $1{\cdot}23 \times 10^{-2}$ sec.$^{-1}$ and $0{\cdot}35 \times 10^{-2}$ sec.$^{-1}$ respectively. In methyl*cyclo*hexane the decay process has an activation energy of 11·4 kcal./mole and a frequency factor of $3{\cdot}1 \times 10^{6}$ sec.$^{-1}$.

These kinetic results show that the phenoxy radical does not decay according to equation (2), since such a reaction would follow second-order kinetics. Furthermore, the first-order kinetics are consistent with the decay being due to an intramolecular rearrangement rather than dimerization or complex formation, which would follow second-order kinetics.

The suggestion that two phenoxy radicals form a complex before rearranging is based on the fact that the cross-products, from the reaction between phenoxy and benzyl radicals, are not formed in appreciable quantities. However, recent results suggest that the *t*-butyl-phenoxy (VI) reacts surprisingly slowly with various other free radicals[4]. Thus it is quite possible that the cross-reactions in the case of the methyl phenoxy (IV) only occur slowly.

The frequency factor is very low for a unimolecular reaction. Dewar[5] has suggested that during an intramolecular rearrangement the migrating group forms a π-complex with the ring, and that bulky substituents on the ring will reduce the rate of rearrangement. In addition, since a free radical possesses an excess electron, it will only form a π-complex with difficulty and thus be rearranged only slowly. Both these effects may be the cause of the low rate of rearrangement found for the methyl phenoxy (IV).

The ethyl phenoxy (V) also decays with first-order kinetics, but at a slower rate than the methyl phenoxy (IV); for example, the rate constant in benzene at 25° C. is $0{\cdot}75 \times 10^{-2}$ sec.$^{-1}$. The first-order kinetics for the ethyl phenoxy (V) show that this also decays by a rearrangement reaction. This is in contrast to the *iso*propylphenoxy ($R \equiv$ *iso*propyl), which decays by a radical–radical reaction, giving second-order kinetics[6].

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[1] Becconsall, J. K., Clough, S., and Scott, G., *Proc. Chem. Soc.*, 308 (1959).
[2] Müller, E., Ley, K., Scheffler, K., and Manat, R., *Chem. Ber.*, **91**, 2682 (1958).
[3] Cook, C. D., Nash, N. G., and Flanagan, H. R., *J. Amer. Chem. Soc.*, **77**, 1783 (1955).
[4] Cook, C. D., and Depatie, C. B., *J. Org. Chem.*, **24**, 1144 (1959). Also unpublished work in this laboratory.
[5] Dewar, M. J. S., "Electronic Theory of Organic Chemistry", 229, 263 (Oxford, 1949).
[6] Cook, C. D., and Norcross, B. E., *J. Amer. Chem. Soc.*, **81**, 1176 (1959).

Decomposition of Methyl Acetylene

Methyl acetylene (propyne) is akin to acetylene in being an endothermic compound the induced decomposition of which will support a flame in the absence of air. The work now reported and a parallel investigation[1] have shown that as in the case of acetylene there is a critical pressure below which a flame is not propagated in pure propyne vapour. This low-pressure limit of propagation has been determined in a cylindrical vessel of internal dimensions 2 in. diameter by 7 in. long mounted vertically; the pressure of the vapour in the vessel was measured by means of a Bourdon gauge with a steel tube. A fuse-wire igniter supported on the vertical axis near the bottom of the chamber was used to ignite the vapour; the wire, a 1-in. length of 0·0135 in. diameter tinned copper, was energized by a 12 volt a.c. supply. Determinations were made at approximately 20° C. and at 120° C. by increasing the pressure of propyne vapour in the vessel until propagation occurred (indicated by a pressure rise) when the igniter was fired.

Dilution tests have been made in the same apparatus, which was then contained in a heated cabinet maintained at 120° C. to prevent condensation of the propyne vapour at the pressures used. Propyne vapour was mixed with nitrogen, methane and ethane respectively; the composition of the mixtures was

Table 1

Temp. (° C.)	Values of low-pressure limit (p.s.i.g.)	Initial total pressure (p.s.i.g.)	Percentage by volume of diluent required to render mixture non-explosible		
			Nitrogen	Methane	Ethane
Approx. 20	50				
120	31				
		100	30	26	15
		200	45	37	23
		300	55	41	26

determined from measurements of the partial pressures of the constituents in the apparatus and checks were made by analysis using a mass-spectrometer. The minimum amount of each diluent required to prevent flame propagation in the mixture at total pressures of 100, 200 and 300 pounds per square inch (gauge) was determined.

The results of the determinations are shown in Table 1.

At the low-pressure limit the dissipation of heat from the flame is so disproportionately large compared with the heat release by reaction that the flame temperature is reduced to a point where the reaction cannot continue and extinction occurs. The heat is dissipated as radiant losses, in heating the combustion products of the flame and any diluents in the flame, and possibly by thermal cracking of the diluents. Thus diluting the flame increases the quantity of heat dissipated within it and limits can occur above the low-pressure limit for pure propyne.

As might be expected from the presence of the methyl group attached to the acetylene molecule the value of the low-pressure limit of propyne is higher than that reported for acetylene in a vessel of the same diameter[2]. An increase of the temperature of the system causes a decrease in the lower limit of flame propagation for propyne, presumably by decreasing the radiation loss from the flame front to the container walls and also by increasing the available enthalpy in the system. When the values of the low-pressure limit from this and a parallel investigation[1] are plotted versus diameter of vessel in which the determinations were made, a graph of the same form as that reported for acetylene is obtained, though the minimum decomposition pressure range reported for acetylene and some substituted acetylenes[2] has not been investigated in this work.

As an inhibitor of the decomposition flame nitrogen is the least efficient of the diluents used, followed by methane and then ethane. This is the order expected on the basis of the combined effect of the molar heat capacities and readiness to decompose with absorption of heat. Thus the hydrocarbons are the more efficient as they have higher specific heats than nitrogen and they absorb heat on cracking; ethane is more efficient than methane as it is less thermally stable[3]. This result is in agreement with previous work[4] where dilution tests were made with acetylene mixtures at total pressures up to 100 pounds per square inch (gauge), which showed that the efficiency of hydrocarbons in inhibiting the reaction increased with increasing molecular weight, and to a much lesser extent with decreasing thermal conductivity. The result is also in agreement with other results for acetylene decomposition at pressures up to 300 pounds per square inch (gauge), where methane appears to be a more efficient inhibitor than nitrogen; no explanation of the results is offered by the authors[5].

The products of the decomposition of propyne are soot and gases. Mass-spectrographic analysis of the cooled gases from both pure and diluted propyne showed them to be hydrogen and methane (the latter up to 30 per cent of the total volume), though unreacted propyne and traces of ethylene and acetylene were found and the diluents from the mixtures. No 'fixing' of the nitrogen as hydrocyanic acid or ammonia previously reported for acetylene and nitrogen mixtures[6] was noted. It is believed that this is because of the low temperature of the reaction (approximately 1,550° C. theoretical temperature for the reaction $C_2H_4 = 3C_{(s)} + 2H_2$, and probably much less than this due to radiation loss from the flame); previous work[7] indicates that free carbon must be available at temperatures above 1,700° C. in the presence of nitrogen and hydrogen in order for hydrocyanic acid to be formed.

The propyne used was approximately 98 per cent pure, the main impurity being the isomer allene.

The work was carried out at the Rocket Propulsion Establishment, Westcott, Bucks. The help and guidance given by Dr. A. R. Hall during the course of the work are gratefully acknowledged; the gas analyses were made by Dr. G. A. Heath.

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[1] Hall, A. R., and Straker, R. A. M. (to be published).
[2] Nelson, H. H., Sixth Symp. (Internat.) on Combustion, 823 (1956).
[3] Egloff, G., and Parish, C. T., *Chem. Rev.*, **19**, 145 (1936).
[4] Jones, G. W., Kennedy, R. E., and Spolan, I., U.S. Bureau of Mines, Report of Investigations, No. 4196 (Jan. 1948).
[5] Hanford, W. E., and Fuller, D. L., *Indust. Eng. Chem.*, **40**, 1171 (1948).
[6] Garner, W. E., and Matsuno, K., *Trans. Chem. Soc.*, **119**, 1903 (1921).
[7] Garner, W. E., and Saunders, S. W., *Trans. Chem. Soc.*, **128**, 1634 (1924).

Alkylation of Coal by Benzyl Chloride–Aluminium Chloride

ALTHOUGH several reports have recently been made[1] on the carbonization of model substances synthesized to imitate the structure of coal, the study of coal pyrolysis has been hampered due to lack of knowledge of the pattern of behaviour of the functional groups. It was therefore decided to alkylate coals with alkylating agents of known structure and study their behaviour on carbonization. This seems to be the first time that alkylation of coal has been reported.

Coal (25 gm.) suspended in carbon disulphide (40 ml.) was heated with benzyl chloride (10 ml.) in the presence of anhydrous aluminium chloride (3 gm.) at 90–100° C. for 2 hr. Under similar conditions benzene is known to be alkylated to diphenyl methane[2]. The product was then hydrolysed by hydrochloric acid and filtered. The residue was repeatedly washed with distilled water until free of acid, and then dried in a vacuum oven.

Elementary analyses of the coals and products are given in Table 1. With the exception of lignite, the coals show an increase in the content of volatile matter. Similarly, except for the high-rank Albion, Jharia and Bhulan Bararee coals, all the coals show an increase in their carbon content. The ratio hydrogen/carbon changes erratically—in some cases increasing, in others decreasing, and in others not showing any change.

Table 1. Alkylation of Coal by Benzyl Chloride–Aluminium Chloride

Coal	Elementary analysis of original coal (dry mineral-free basis per cent)							Elementary analysis of alkylated coal (dry mineral-free basis per cent)						
	V.M.	C	H	N	S	O	Atomic H/C	V.M.	C	H	N	S	O	Atomic H/C
1 South Arcot lignite	54·0	69·0	4·9	0·6	1·1	24·4	0·86	53·9	73·5	4·6	0·6	0·7	20·6	0·76
2 Jambad Bowlah	39·0	77·9	5·2	1·9	0·4	14·6	0·80	49·1	82·7	5·7	1·2	0·3	10·1	0·83
3 North Chora vitrain	35·5	78·7	5·0	2·3	0·8	13·2	0·75	—	79·1	5·0	2·0	0·7	13·6	0·75
4 Dishergarh	41·8	83·8	5·7	2·3	0·4	7·8	0·82	43·0	85·1	5·6	1·9	0·3	7·1	0·78
5 Seetalpur vitrain	33·5	82·9	5·1	2·1	0·5	9·4	0·73	—	82·8	5·2	1·7	0·9	9·4	0·75
6 Bhulan Bararee	31·5	86·5	5·0	1·9	0·5	6·1	0·70	38·0	86·9	5·1	1·4	0·4	6·2	0·72
7 Albion Jharia vitrain	—	87·6	4·8	2·2	0·5	4·9	0·66	36·2	87·9	4·9	2·2	0·5	4·6	0·68

Table 2. Low-Temperature Carbonization of Alkylated Coal

Coal	Original coal (dry mineral-free basis per cent)				Alkylated coal (dry mineral-free basis per cent)			
	Coke	Tar	Liquor	Gas	Coke	Tar	Liquor	Gas
1	42·2	13·4	28·1	17,375	61·2	19·1	6·7	12,120
2	70·5	13·0	6·7	—	60·3	25·3	6·6	10,310
3	72·8	8·3	7·1	11,320	65·8	16·3	10·8	8,970
4	73·0	12·4	8·5	10,670	68·0	19·3	5·0	9,390
5	79·3	8·4	4·6	11,200	71·0	15·0	6·6	7,250
6	80·2	7·3	3·2	9,930	74·6	15·8	—	9,930

A careful study of the ratio hydrogen/carbon in alkylated models of polycondensed aromatic systems shows that it depends on the number of aromatic rings present in the system. For example, benzene, naphthalene, and pyrene exhibit a decrease in the ratios hydrogen/carbon on alkylation, whereas a 10-membered system indicates an increase in the hydrogen/carbon ratio on alkylation. But in the case of coal, it is not clear whether or not the variation in the hydrogen/carbon ratio is attributable to the number of rings in the aromatic system. X-ray results with treated coals have indicated marked variation in the size and distribution of the aromatic nucleus compared to original coals (Ali, S. Z., private communications).

The most significant changes are in the carbonization assays (Table 2). In all cases there is considerable increase in the yield of tar, which is nearly doubled. Except for lignite, the coals show a decrease in the coke yield. In the case of lignite there is a marked decrease in the liquor yield and also in the coke yield. But in the other cases there is no regularity about the variation in the liquor. There is also a deterioration in the quality of coke. For example, Bhulan Bararee coal gave originally a G_1 type coke, but after treatment only a C type coke. In all cases there is a decrease in yield of gas.

There is no doubt that coal reacts with benzyl chloride and that this reaction considerably influences the yield of tar, and the quality of coke. A detailed consideration of the structural changes on alkylation and the changes on carbonization will be published later.

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[1] Kipling, J. J., and Wilson, R. B., Proc. Cong. Science in the Use of Coal, Inst. Fuel, C-8 (1958). Wolfs, P. M. J., Van Krevelen, D. W., and Waterman, H. I., Third Int. Conf. Coal Science, Netherlands (1959). Depp, E. A., Sievens, C. M., and Neuworth, M. B., *Fuel* (Lond.), **35**, 437 (1956). Depp, E. A., and Neuworth, M. B., Proc. Second Int. Conf. Coal Science, *Brennstoff-Chemie*, **39**, 590 (1958).

[2] Adams, R., Bachman, W. E., Fieser, L. F., Johnson, J. R., and Snyder, H. R., "Organic Reactions", **3**, 38 (John Wiley and Sons, New York, 1947).

Acidic Dissociation Constant of Alcoholic Hydroxyls of Oxycarboxylic Acids: Mandelic Acid

The oxycarboxylic acids are very strong complex-forming agents, and both the carboxylic and the alcoholic hydroxyl groups take part in such formation. For calculation of complex stability constants it is necessary to know the acidic dissociation constants of both groups. However, the alcoholic hydroxyls are so weakly acidic that it is not possible to determine the dissociation constant by the usual methods. So far as we know, until now none of the oxycarboxylic acids has been investigated successfully from the point of view of acidity of alcoholic hydroxyls. The only data which we found referred to citric acid; however, these were theoretical values[1].

In general, the oxycarboxylic acids show no absorption even in the extreme ultra-violet regions. An exception is the absorption of mandelic acid, which, due to the benzene ring, is fairly high in the ultra-violet region. However, absorptions of mandelic acid and the mandelate ion do not differ from each other. We determined the change of absorbancy at very high *p*H, that is to say, in strongly alkaline solution.

The mandelic acid shows a maximum absorption at 264 mμ and a minimum at 244 mμ. The effect of ionizing the carboxyl group is to increase the maximum to 260 mμ; however, the minimum does not change. The effect of greater alkali concentration (increasing ionization of alcoholic hydroxyl) causes both the maximum and the minimum to disappear; the absorption monotonously increases with decreasing wave-length.

To calculate the dissociation constant we assumed that the only reason for the change in absorption on treatment with sodium hydroxide is the gradual ionization of alcoholic hydroxyl. Table 1 shows the change of absorption at 244 mμ as a function of *p*H. We used the *p*H values determined by Schwarzenbach and Sulzberger[2] instead of sodium hydroxide concentration. The absorption does not reach a limiting value due to the equilibrium:

$$\underset{\text{OH}}{\text{C}_6\text{H}_5\text{CH}\cdot\text{COO}^-} \rightleftharpoons \underset{\text{O}^-}{\text{C}_6\text{H}_5\cdot\text{CH}\cdot\text{COO}^-} + \text{H}^+$$

(I) (II)

being shifted so far to the right even at maximal sodium hydroxide concentration that practically all the mandelic acid is in the form of ion II. Thus the molar absorption of II cannot be calculated directly and hence we calculated it by Stearns and Wheland's method[3]. The molar absorption of I at 244 mμ is 120·5, while that of II is 719 at the same wave-length. Using these values the concentrations of I and II in the dissociation constant :

$$K = \frac{[\text{II}]\ [\text{H}^+]}{[\text{I}]}$$

can be calculated. The values of K are given in the fourth column of Table 1, its average being $7{\cdot}19 \times 10^{-16}$.

Table 1

[NaOH]	pH	A_{244}	$K \times 10^{16}$
—	4	0·250	—
—	10	0·236	—
0·279	13·3	0·250	6·75
1·395	14·1	0·344	8·24
2·79	14·5	0·477	8·29
5·58	15·2	0·747	4·45
10·16	16·2	1·315	7·96
		Average	7·19

Details of this research will be published in full elsewhere. Thanks are due to Dr. J. Császár for the measurements of absorption.

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[1] Warner, R. C., and Weber, I., *J. Amer. Chem. Soc.*, **75**, 5086 (1953).
[2] Schwarzenbach, G., and Sulzberger, R., *Helv. Chim. Acta*, **27**, 348 (1944).
[3] Stearns, R. S., and Wheland, G. W., *J. Amer. Chem. Soc.*, **69**, 2025 (1947).

BIOCHEMISTRY

Structure of Bhilawanol

Bhilawanol is the golden yellow liquid found in the pericarp of the marking nut *Semecarpus anacardium*. It forms a di-naphthylurethane and a dimethyl ether and on catalytic reduction it absorbs one mole of hydrogen to give hydrourushiol (3-pentadecylcatechol)[1,2].

Ozonolysis of the diphenylmethylene ether gave heptaldehyde[2] (in low yield), suggesting that at least a portion of the bhilawanol is 3-(pentadecenyl-8′)-catechol (thus corresponding to the urushenol of poison ivy and Japanese lac[3]).

Although distillation of dimethyl bhilawanol[1] and chromatography of the diphenylmethylene ether[2] pointed to bhilawanol as being a single substance, chromatography of the dimethyl bhilawanol using the procedure described by Symes and Dawson[4] showed that it was not homogeneous (Symes, W. F., unpublished work). Furthermore, the glycol, m.p. 56–60°, prepared by performic acid hydroxylation of distilled dimethyl bhilawanol, on chromatographing, separated into two glycols, one melting at 66·8°, the other at 93–4° (Symes, W. F., unpublished work).

The low-melting isomer is identical to the glycol prepared by performic acid (*trans*) hydroxylation of the dimethyl ether of the stereochemically pure *cis* poison ivy urushenol[4]. The high-melting isomer is identical to the glycol prepared by osmium tetraoxide (*cis*) hydroxylation of this same material[3]. It therefore becomes clear that the bhilawanol is a mixture of the *cis* and *trans* isomers of urushenol.

These conclusions are evident from Table 1.

Table 1

Compound	n_D^{25}	m.p.	Dimethyl ether		Di-naphthyl-urethan, m.p.
			n_D^{25}	Glycol, m.p.	
Bhilawanol*	1·5032 (ref. 1)	15°	1·5000	67 and 93†	138–40° (ref. 1)
Urushenol (*cis*)‡	1·5081	33°	1·4945	95† 67§	139–41° (ref. 3)
Urushenol (*cis–trans*) (ref. 3)	1·4970	10–15°		92° and lower melting solid†	137–39° (ref. 3)

* Distilled but not chromatographed ; † performic acid hydroxylation ; ‡ synthetic (ref. 3) ; § osmium tetraoxide hydroxylation.

The melting ranges of the solid derivative, the dinaphthyl urethane, is evidently a poor criterion of homogeneity in this series.

Bhilawanol and the *cis–trans* urushenol prepared by acid catalysed isomerization of *cis* urushenol[3] are very similar, and there can be little doubt that bhilawanol contains a component identical to the monolefinic component of poison ivy urushenol.

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[1] Pillay, P., and Siddiqui, S., *J. Indian Chem. Soc.*, **8**, 1517 (1931).
[2] Mason, H. S., *J. Amer. Chem. Soc.*, **67**, 418 (1945).
[3] Loev, B., and Dawson, C. R., *J. Org. Chem.*, **24**, 980 (1959).
[4] Symes, W. F., and Dawson, C. R., *J. Amer. Chem. Soc.*, **76**, 2959 (1954).

Age Difference in Nicotinamide Mononucleotide Synthesis by Human Erythrocytes

It is well known that human erythrocytes contain an active enzyme system capable of synthesizing considerable amounts of nicotinamide mononucleotide from nicotinamide added *in vitro*[1]. The nicotinamide mononucleotide synthetase is very specific for human erythrocytes as the only other mammal in the blood cells of which it could be detected is the guinea pig[2]. It was found here, however, that the activity of the given synthetase in human erythrocytes can be shown at the beginning of the fifth month of life when its activity rapidly reaches almost a maximum level, remaining then practically without change. Whereas mammalian erythrocytes not synthesizing nicotinamide mononucleotide are characterized by a high content of pyridine nucleotides, their amount being several times higher than in human and guinea pig erythrocytes[2], the non-synthesizing child erythrocytes contain even somewhat less pyridine nucleotides than those of adult humans.

The amount of pyridine nucleotides and the nicotinamide mononucleotide synthetase activity were determined fluorometrically by a slightly modified method of Leder and Handler[1]. The nicotinamide mononucleotide synthetase activity was expressed as :

$$I_{20h} = \frac{b - a}{a}$$

where a is the concentration of pyridine coenzymes in μmoles/ml. erythrocyte mass before incubation and b the same after 20 hr. stationary incubation at 37° C. in Krebs–Ringer phosphate solution containing 250 mgm. per cent glucose and 2 per cent nicotinamide besides the mineral constituents. A total of 80 samples of heparinized blood from quite normal children and adults was examined. The results are shown in Fig. 1.

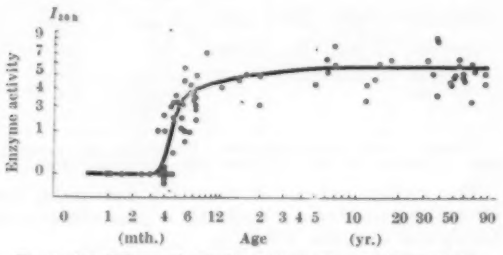

Fig. 1. Age difference in nicotinamide mononucleotide synthetase activity in human erythrocytes

The erythrocyte synthetase activity of adult humans under the given conditions amounts to 4·8 ± 1·3. Marked changes are observed in some diseases; for example, the activity is considerably decreased or completely lost in uræmia (a communication on this point will be presented later). The content of pyridine nucleotides in adult human erythrocytes is 0·11 ± 0·01 μmole/ml. erythrocyte mass, erythrocytes of infants before 4 months of age 0·09 ± 0·01 μmole/ml. The pyridine coenzyme-level of erythrocytes is very constant and is not affected by those factors which change markedly the nicotinamide mononucleotide synthetase activity *in vivo*.

The basis of inability of sucklings' erythrocytes to synthesize nicotinamide mononucleotide has not yet been elucidated. It is possible that erythrocytes produced in the course of the first months of life, when the synthesis of fœtal hæmoglobin regresses, are not mature in this respect; it cannot be excluded, however, that a specific or a non-specific inhibitor of the given synthetase was present.

However, the results present a further proof that human biochemical ontogenesis is not limited to the embryonic and fœtal period. Some metabolic systems develop and improve only after birth; the first months of life appear to be of exceptional importance in this respect.

We are grateful to Dr. M. Lukešová, Clinic of Pediatry, and to Dr. V. Čepelák, Medical University Clinic, for supplying us with the blood samples. We also wish to thank Miss E. Maidlová for technical assistance.

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[1] Leder, I. G., and Handler, P., *J. Biol. Chem.*, **189**, 889 (1951).
[2] Tulpule, P. G., *Nature*, **181**, 1804 (1958).

Microbiological 11β-Hydroxy Dehydrogenation of Corticosterone

A large number of bacteria are known to dehydrogenate the secondary alcohol group of the steroid molecule, at carbon 3 and 17 [1], and also in position 7 and 12 [2].

However, dehydrogenation of the 11β-hydroxy group by micro-organisms has been reported in a few cases. For example, *Cunninghamella blakesleeana*[3] produced a certain amount of cortisone as a by-product of hydrocortisone from Reichstein's compound *S*, and *Trichothecium roseum*[4] produced a small amount of cortisone besides hydrocortisone from corticosterone. From these examples, it appears that 11β-hydroxy dehydrogenase, which has been known to be present in mammalian tissues, is also present in micro-organisms.

During our work on microbiological oxidation of steroids, we found the effective dehydrogenation of the 11β-hydroxy group of corticosterone by *Absidia regnieri*. In this case, introduction of a hydroxy group in the 14α position of the steroid molecule was caused by this micro-organism.

The present communication reports the effective dehydrogenation of the 11β-hydroxy group of corticosterone. In our experiment, *Absidia regnieri* was grown on a reciprocating shaker for 48 hr. at 27° in 5 l. of a medium of the following composition: 5 per cent glucose, 2 per cent peptone and 0·5 per cent corn steep liquor. Then 2·5 gm. corticosterone dissolved in methanol was added in this culture and fermentation was allowed to proceed for an additional 48 hr. At the end of fermentation, the culture was separated into mycelium and liquid, and these were extracted with ethyl acetate ester, respectively. The combined ethyl acetate ester extract was evaporated to dryness *in vacuo*. Paper partition chromatography of this residue showed only one oxygenated product and no unreacted steroid. The oxygenated product was isolated by direct crystallization or chromatography of 'Florisil'.

Crystallization of this residue with acetone afforded a 14α-hydroxy-11-dehydrocorticosterone (I); m.p. 205–215°; $[\alpha]D$ + 69° (methanol); $\lambda_{max.}^{MeOH}$ 237·5 mμ (ε = 15,000); $\lambda_{max.}^{KBr}$ 3,480, 1,705, 1,660 and 1,620 cm.$^{-1}$; anal. found: C, 69·70; H, 7·78 per cent; calc.: C, 69·97; H, 7·83 per cent. 21-Monoacetate of I was prepared with pyridine and acetic anhydride; m.p. 204–5°; $\lambda_{max.}^{MeOH}$ 237 mμ (ε = 15,300); $\lambda_{max.}^{KBr}$ 3,560, 1,750, 1,735, 1,690 and 1,625 cm.$^{-1}$.

The structure of I was established in the following manner. When 11-dehydrocorticosterone was fermented with this fungus, the oxygenated product obtained was identical to I. This result and the observed ultra-violet maximum 237 mμ of I indicate that the 11β-hydroxy group of the steroid was converted to ketone. To decide the position of the new hydroxy group introduced, we have prepared new steroid 14α-hydroxy-corticosterone (II) by incubation of known 14α-hydroxy-deoxycorticosterone with *Stachylidium bicolar*, which is capable of 11β-hydroxylating of steroid. The compound II melted at 206–215°; $[\alpha]D$ + 180° (methanol); $\lambda_{max.}^{MeOH}$ 241·5 mμ (ε = 16,500); $\lambda_{max.}^{KBr}$ 3,400, 1,710, 1,650 and 1,620 cm.$^{-1}$. The 21-acetate of II prepared with pyridine and acetic anhydride was oxidized with chromium trioxide-pyridine to 11-ketone. The material thus

obtained agreed with the 21-monoacetate of I by physical constants and infra-red spectrum. This result indicates that the newly introduced hydroxy group must be 14α-hydroxyl.

From these results we conclude that the structure of I is 14α-hydroxy-11-dehydrocorticosterone. This fungus, however, had no effect on the conversion of epicorticosterone and hydrocortisone. This fact shows the marked stereospecificity of this dehydrogenase, that is, it acts only on 11β-hydroxyl of corticosterone and not on that of hydrocortisone or 11α-hydroxyl of epicorticosterone.

With the other steroid substrate, this fungus also showed remarkable substrate specificity as reported previously[6]. That is, it hydroxylated progesterone or deoxycorticosterone at the 14α-position and 17α-hydroxyprogesterone or Reichstein's compound *S* at the 11α-position.

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[1] Talalay, P., and Dobson, M. M., *J. Biol. Chem.*, **205**, 823 (1953). Turfitt, G. E., *Biochem. J.*, **40**, 79 (1946), etc.
[2] Hoehn, W. M., Schmidt, L. H., and Hughes, H. B., *J. Biol. Chem.*, **152**, 59 (1944).
[3] Hanson, F. R., Mann, K. M., Nielson, E. D., Anderson, H. V., Brunner, J. N., Colingsworth, D. R., and Haines, W. J., *J. Amer. Chem. Soc.*, **75**, 5369 (1953).
[4] Meyster, Ch., Vischer, E., and Wettstein, A., *Helv. Chim. Acta*, **37**, 1548 (1954).
[5] Shirasaka, M., Hayashi, R., and Tsuruta, M., *Bull. Agric. Chem. Soc. Japan*, **23**, 244 (1959).

Enhancement of Peroxidase Action by Polysaccharides

A number of polysaccharides act as polymerization-directing matrices in peroxidase-catalysed eugenol oxidations[1]. Although deoxyribonucleic acid and cellulose increase markedly the rate of Fe(III)-catalysed pyrogallol oxidation by hydrogen peroxide[2,3], a similar test of polysaccharides has not been reported previously in peroxidase-catalysed reactions.

The present results show that the catalytic activity of peroxidase is increased appreciably by moderately low concentrations of starch and is somewhat influenced by methylcellulose as well.

Oxidations were followed at 25° C. with the Bausch and Lomb 'Spectronic 20' spectrophotometer in 10-ml. reaction mixtures containing 5 μgm. Worthington horseradish peroxidase, 10^{-2} *M* hydrogen peroxide, substrate and *M*/20 phosphate buffer, *p*H 4·5. Oxidation of 5×10^{-3} *M* pyrogallol was measured at 425 mμ; oxidation of 5×10^{-4} *M* eugenol was measured at 360 mμ.

The chemical substances used were of reagent grade when possible. Methylcellulose solutions were prepared from 'Dow Methocel *HG* 400'. Neither starch nor methylcellulose exhibited catalytic activity when tested directly with hydrogen peroxide and pyrogallol.

In ten experiments, 5×10^{-5} *M* starch increased oxidation of pyrogallol by peroxidase appreciably (Table 1). The initial rate (0·5 min.) was most affected, but considerable enhancement is still in evidence after 2–3 min., when the control reaction-rate has slowed markedly.

Table 1. Effect of Starch on Peroxidase-catalysed Pyrogallol Oxidation*

Reaction time (min.)	(*a*) Control: Average optical density	(*a*) Control: σ	(*b*) Starch*: Average optical density	(*b*) Starch*: σ	*b*/*a* (percentage control)
0	0	—	0	—	—
0·5	0·098†	± 0·018	0·153	± 0·016	156
1·0	0·254	± 0·030	0·373	± 0·019	147
1·5	0·437	± 0·036	0·597	± 0·023	137
2·0	0·635	± 0·036	0·847	± 0·023	133
3·0	0·775	± 0·062	1·033	± 0·047	133

* Reaction mixture: 10 ml. *M*/20 phosphate, *p*H 4·5, containing 5×10^{-6} *M* pyrogallol, 10^{-2} *M* hydrogen peroxide, 5 μgm. peroxidase, with or without 5×10^{-5} *M* starch. Oxidation was carried out at 25° C. and measured at 425 mμ.
† Each figure is the mean of 10 determinations.

Stimulation is not limited to the oxidation of pyrogallol, but is also obtained with eugenol as substrate (Table 2*b*).

Table 2. Effect of Several Carbohydrates on the Peroxidation of Pyrogallol and Eugenol*

Carbohydrate	Concentration (*M*)	Effect (percentage control after 1 min.)
	(*a*) Substrate: pyrogallol	
Glucose	5×10^{-5}	100
	10^{-3}	99
	0·5	84
Maltose	5×10^{-5}	103
	10^{-3}	100
	0·5	86
Methylcellulose	5×10^{-5}	117
Starch	5×10^{-5}	147
	(*b*) Substrate: eugenol	
Glucose	0·5	83
Methylcellulose	5×10^{-5}	125
Starch	5×10^{-5}	143

* Reaction conditions as given in Table 1 except that eugenol was used at 5×10^{-4} *M* and its oxidation followed at 360 mμ.

Methylcellulose is not as stimulating as starch when tested with either substrate, but the activity of these two macromolecules may be contrasted with the effects of glucose and maltose (Table 2*a*, *b*). The simple sugars have no effect in low concentrations, and they are slightly inhibitory at high concentrations. A proper comparison between simple sugars and polysaccharides cannot be made on a mole-for-mole basis, nor can they be equated in a simple way by comparing the number of monosaccharide residues present.

A macromolecular basis for the stimulatory effects of polysaccharides is suggested by the absence of enhancement of sugars over a wide range of concentrations. Possibly the polysaccharides provide a concentrating surface, hence increasing the probability of reaction, and render adsorbed peroxidase more efficient as a catalyst. These suggestions receive support from the greater effect of starch on pyrogallol oxidation at lower peroxidase concentrations. Thus, with 0·5 μgm. of enzyme, an initial simulation (0·5 min.) of 100 per cent was observed, declining to 50 per cent after 3 min.

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[1] Siegel, S., in "Sub-cellular Particles", edit. by Hayashi, T., (Amer. Physiol. Soc., Washington, 1959).
[2] Siegel, S., and Siegel, B. Z., *Nature*, **179**, 421 (1957).
[3] Goodman, N. S., and Siegel, S., *Nature*, **184**, 53 (1959).

Starch–Agar-Gel Electrophoresis of Tuberculoproteins from Heated Culture Filtrates

In order to improve the specificity of a bovine tuberculin purified protein derivative, precipitated with trichloracetic acid from heated culture filtrates[1], we decided to investigate its protein composition[2], and in this connexion we experimented with zone electrophoresis on paper-strips. Now this procedure has hitherto not been very successful[3–5], and in spite of our own numerous experiments with various procedures and methods we did not obtain anything other than unusable pictures of 'comets' or streaks. (This is in contrast with the more satisfying results obtained by others with non-heated tuberculins[4,5].) Smithies introduced starch gel electrophoresis which seems to be of great value in determining the ultrastructure of serum-protein patterns[6,7], the composition of toxins[8] and of cerebrospinal fluid[9]. Sorkin *et al.*[10] made preparative separations of some tuberculoprotein preparations from unheated culture filtrates by electrophoresis in starch gels, followed by transfer into glass. Our own investigations on this starch-gel electrophoresis led to the following method.

In order to prevent premature desiccating of the gels and to avoid the necessity of protecting them by placing covers and/or mineral oils on the surface and still to maintain a satisfactory consistency, we used 10 per cent w/v 'Merck Amylum Solubile' (*A.R.*) plus 0·27 per cent w/v 'Difco Bacto' agar. The agar was at first weakened for about 30 min. or longer in the buffer solution consisting of 15 gm./l. boric acid, 0·75 gm./l. bi-sodium salt of ethylenediamine tetraacetic acid and 2·5 mgm./l. 'Tween 80', brought up to *p*H 7·5 by adding about 6 gm./l. *tris* hydroxymethyl aminomethane. The mixture was then boiled until the agar was dissolved. After some time of cooling the solution, the starch was added, suspended, heated with continuous stirring and finally boiled for a few seconds. The liquid gel was poured out in plastic ('Perspex') trays to form layers of 240 mm. × 60 mm. × 3 mm., the trays having been placed in an electrophoresis apparatus with platinum electrodes. The electrode vessels and bridge vessels were filled with the above-mentioned buffer, only without 'Tween 80', and with sodium hydroxide instead of *tris* buffer. Thick filter-paper strips formed the connexion between bridge vessels and tray. After about 20 min. we made an incision, 8 cm. from the anodic side of the tray, with a thin blade and allowed 0·05–0·07 ml. of a protein solution of about 60 mgm./ml. to drip down slowly into the incision, even when it was scarcely visible on the surface. Immediately afterwards the gels were subjected to electrophoresis at room temperature for 17 hr. at 6 V./cm., then left to stain with Bayers 'Amidoschwarz 10 *B*' (2 gm./l. solution of methanol/water/acetic acid, 45 : 45 : 10) for 15 min., and washed with the same solution over a period of 1 hr., and repeated about 5 times with fresh solution. Fig. 1 shows the patterns of our bovine purified protein derivative tuberculin, solved from the lyophilized state by dialysing against a 0·25 per cent phenolic diluted buffer of *p*H 7 until the required protein concentration was reached; the '*C*'-like protein fraction, prepared from the same purified protein derivative, fundamentally following Seibert's method[11] and the '*A* + *B*'-like proteins, prepared from the (purified protein derivative minus '*C*') remainder.

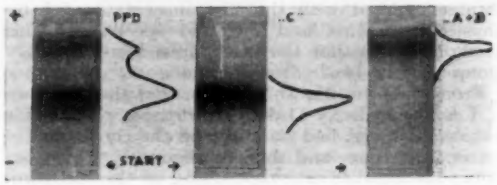

Fig. 1. The curves were obtained from colour diapositives of the patterns by photoelectric scanning

Full details of these preparations will be described in a publication elsewhere.

It appears from the purified protein derivative diagram that the proportion '*A* + *B*'/'*C*', calculated from planimetric measurement, is about a third, which is in accordance with the quantities of the chemically separated fractions. The '*C*'-like protein, its molecular weight probably being twice as great as that of the unheated '*C*'[12], is the slower one, in this respect quite the reverse of the unheated '*C*' in the Tiselius picture[3]. Furthermore, our '*C*'-like protein shows a greater tendency towards tailing than the '*A* + *B*'-like fraction.

These last two proteins appear to move closely together. *p*H's higher than 7·5 tend progressively to suppress separation while the lower ones tend to sharpen the zones but increase adsorption to the gel substance. Our results point to the combined effects of electric charge and particle size in starch-gel electrophoresis[13].

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[1] Dekker, T., and Hultema, H., *Nature*, **182**, 1387 (1958).
[2] Seibert, F. B., *Amer. Rev. Tuberc.*, **59**, 86 (1949).
[3] Ribeiro, J. M., and Fernandes, A. A. S., *Bull. Int. Off. de l'Epiz.*, **42**, 321 (1954).
[4] Rhodes, J. M., and Sorkin, E., *Experientia*, **10**, 427 (1954).
[5] Sorkin, E., and Rhodes, J. M., *Helv. Chim. Acta*, **39**, 1539 (1956).
[6] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[7] Ashton, G. C., *Nature*, **179**, 824 (1957).
[8] Poulik, M. D., *Nature*, **180**, 1479 (1957).
[9] Pert, J. H., and Kutt, H., *Proc. Soc. Exp. Biol. and Med.*, **99**, 181 (1958).
[10] Sorkin, E., Rhodes, J. M., and Boyden, S. V., *Helv. Chim. Acta*, **39**, 1547 (1956).
[11] Seibert, F. B., Figueroa, E. S., and Dufour, E. H., *Amer. Rev. Tuberc.*, **71**, 704 (1955).
[12] Takeya, K., and Toda, T., *Enzymologia*, **19**, 65 (1958).
[13] Martin, N. H., *Analyst*, **84**, 93 (1959).

Effect of Pituitary Growth Hormone *in vivo* on the Phosphorus/Oxygen Ratios of Isolated Rat Liver Mitochondria

Recent reports tending to localize the site of action of thyroxine in uncoupling oxidative phosphorylation at the level of the mitochondrial membrane[1,2] have served to emphasize the need for similar studies on possible sites of action of anterior pituitary growth hormone, a substance which produces generalized metabolic changes even more striking than those found with thyroxine. The present communication reports some results concerning the effect of the hormone on oxidative phosphorylation, using mitochondria isolated from the livers of normal rats or rats treated with growth hormone.

Rats were injected 4 hr. before killing either with 1 mgm. purified beef growth hormone or with 1–5

Table 1. PHOSPHORUS/OXYGEN RATIOS OF MITOCHONDRIA ISOLATED FROM THE LIVERS OF NORMAL AND GROWTH HORMONE-TREATED RATS

Treatment	No. of animals	μmoles acetoacetate found	μmoles glucose-6-phosphate formed	Phosphorus/oxygen ratio
Control	5	0·240 ± 0·030	0·652 ± 0·072	2·71 ± 0·058
Growth hormone	5	0·337 ± 0·032	0·651 ± 0·049	1·93 ± 0·050
Albumin	3	0·260 ± 0·037	0·675 ± 0·095	2·64 ± 0·073
Fisher's 't' growth hormone v. controls		0·03	not significant	< 0·001

1 mgm. of growth hormone or 5 mgm. of bovine plasma albumin (Armour) injected 4 hr. before killing.
System. 0·3 ml. 0·3 *M* glucose; 0·1 ml. 0·04 *M* adenosine diphosphate; 0·1 ml. 3 × 10^{-4} *M* cytochrome *c*; 0·5 ml. 0·1 *M* phosphate buffer *p*H 7·4; 0·1 ml. 0·2 *M* magnesium sulphate; 0·05 ml. 1·5 *M* potassium chloride; 0·1 ml. 0·1 *M* versene *p*H 7·4; 0·3 ml. 0·1 *M* *dl*-β-hydroxybutyrate acid; 0·05 ml. hexokinase solution containing 50 units of enzyme activity; 0·2 ml. bovine plasma albumin (7·5 mgm./ml.); 0·2 ml. 0·02 *M* sodium fluoride and 1 ml. liver mitochondria (8 per cent suspension).
Temperature 27°. Time 5 min.

mgm. bovine plasma albumin. After killing, the livers were rapidly removed, the mitochondria isolated in 0·25 *M* sucrose and the phosphorus/oxygen ratios measured using a system adapted from that of Maley[3] with either *d*-, *l*-, or *dl*-β-hydroxybutyrate, *dl*-glutamate or reduced diphosphopyridine nucleotide as substrate.

Under the conditions employed here *d*-β-hydroxybutyrate was not oxidized at all, since *l*-β-hydroxybutyrate gave results exactly comparable with those obtained using the *dl*-salt. It will be seen that the mitochondria from growth hormone-treated rats have a lower phosphorus/oxygen ratio than those from normal rats, and that this difference can be largely ascribed to an increase in the rate of oxygen utilization rather than to any decrease in the rate of phosphorylation. The one-step oxidation of β-hydroxybutyrate involves the production of reduced diphosphopyridine nucleotide, and it is this latter compound which acts as substrate for the oxidative phosphorylation[4]. For this reason, the efficiency of the phosphorylation accompanying the oxidation of glutamate, an alternative source of reduced diphosphopyridine nucleotide, and diphosphopyridine nucleotide itself were studied. The phosphorus/oxygen values using these two substrates were lower than the values recorded for β-hydroxybutyrate (2·31 for glutamate and 1·77 for reduced diphosphopyridine nucleotide) and were entirely unaffected by prior treatment of the animal with growth hormone. Thus the ability of growth hormone to affect the efficiency of oxidative phosphorylation could only be demonstrated in one of the three enzyme systems tested, that is, β-hydroxybutyrate dehydrogenase and its associated electron transporting chain. It is interesting to notice that thyroxine also uncouples oxidative phosphorylation when β-hydroxybutyrate is used as substrate[1] although it should be observed that in this latter case the mechanism appears to be somewhat different from that shown by growth hormone (Table 1) in that thyroxine does not affect the oxygen uptake but causes a decrease in the associated phosphorylation.

In view of the widespread metabolic effects of growth hormone *in vivo* it would seem unlikely that the hormone has a specific effect on β-hydroxybutyrate dehydrogenase alone. Of the three substrates tested, only β-hydroxybutyrate seems to be oxidized exclusively by an 'internal pathway' (that is, one inside the mitochondrion) and since this enzyme is also the only one affected by prior treatment of the animal with growth hormone it would appear probable that the site of action of the growth hormone in lowering the efficiency of oxidative phosphorylation is at the level of the mitochondrial membrane, as has been suggested for thyroxine[1,2].

It is difficult to visualize a means by which a lowering of the efficiency of oxidative phosphorylation can be of advantage to an animal; but perhaps the lowered phosphorus/oxygen ratio is of secondary importance to the change in mitochondrial permeability. Ernster[5] has suggested that a reversible swelling of the mitochondria could provide a transfer system by which there could be an interchange of materials between the mitochondrial interior and cytoplasm. If the action of growth hormone is to alter the mitochondrial permeability then it is possible to consider that this effect of the hormone could, by permitting a transfer of metabolites or co-factors across the membrane, lead to increased metabolic activity which, even if there were some loss of efficiency in the coupling of some of the reactions, would be to the overall advantage of the animal. The increased rate of oxidation of β-hydroxybutyrate by the hormone-treated animal appears to point to such a possibility.

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[1] Tapley, D. F., and Cooper, C., *J. Biol. Chem.*, **222**, 325 (1956).
[2] Lehninger, A. L., Ray, B. L., and Schneider, M., *J. Biophys. Biochem. Cytol.*, **5**, 97 (1959).
[3] Maley, G. F., *J. Biol. Chem.*, **224**, 1029 (1957).
[4] Lehninger, A. L., *J. Biol. Chem.*, **164**, 291 (1946).
[5] Ernster, L., *Exp. Cell Res.*, **10**, 721 (1956).

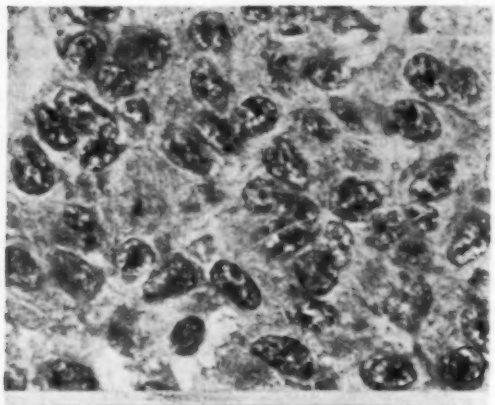
PHYSIOLOGY

Effects of Injection of Bovine Pineal Extract on the Nuclei of Rat Pineal Parenchymal Cells

THE pineal parenchymal cells are known to be rich in nucleoprotein[1,2]. Oksche[3] showed that pineal-cell nuclei contain a 'nucleolus apparatus' which extrudes some nuclear substance into the cellular cytoplasm. In general, the number of nucleoli and intranuclear granules in any cell is probably a useful index of the rate of protein synthesis[4]. This idea has led several authors to use pineal intranuclear changes as evidence of changed pineal functional status: both Oksche[3] and Quay[5] reported variations in pineal intranuclear activity owing to changes in illumination of their animals' cages. Quay[5] also correlated changes in pineal cell nuclei with periodic variations in pituitary gonadotropic activity.

The present communication describes changes in pineal nuclear status resulting from injections of a pineal extract. The occurrence of such changes suggests that exogenous pineal extract depresses pineal function in intact animals.

Studies were made on pineal glands from 15 Charles River rats which had been given intraperitoneal injections of a bovine pineal extract daily from the ages of 21–49 days; these were compared with the glands of control animals given saline injections.



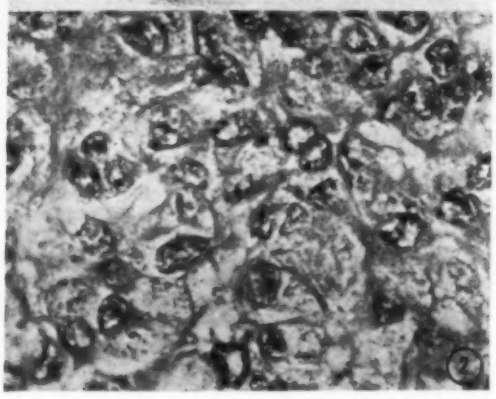


Fig. 1. The pineal parenchymal cells of the rat. Gomori's chrome hæmatoxylin–phloxin counter-stained in tartrazine (× *c.* 950).
Fig. 2. The pineal parenchymal cells of a pineal-treated rat. Notice the decrease in number of nucleoli and in the amount of intranuclear material; the central portions of the nuclei are completely clear in many cases. The cytoplasm is also paler and less granular than in the control animals. Gomori's chrome hæmatoxylin–phloxin counter-stained in tartrazine (× *c.* 950)

The bovine pineal extract was prepared essentially according to the method described elsewhere[6].

The pineal glands were fixed in Bouin's solution to which 1 per cent calcium chloride had been added; they were stained with Gomori's chrome hæmatoxylin–phloxin and in phloxin and then counterstained with tartrazine according to Lendrum[7]. (The treatment with hæmalum was omitted.) The sections were cut at 3–4μ. The number of phloxinophilic nucleoli above approximately 0·5μ in diameter were counted.

The pineal parenchymal cells of the rats which had been given pineal extract showed a marked decrease in the number and size of the nucleoli and the amount of granular material in the nuclei (Figs. 1 and 2). The decrease in number of nucleoli in the pineal-treated animals was statistically significant ($P < 0{\cdot}001$). A slight decrease in the size of the pineal cells themselves was noted in the glands of the treated animals; the cytoplasm appeared less dense than in the control animals.

Caspersson[4] showed that a decrease in the number of nucleoli and intranuclear granules in cells indicated a reduced rate of nuclear synthesis. The decrease in the intranuclear granular and nucleolar material observed here in rats given a bovine pineal extract must therefore indicate that such extracts depress nuclear activity in the pineal cells of injected animals. Secretory activity in the pineal cell is known to be correlated with nuclear activity[3]. The reduced pineal nuclear activity found here therefore suggests that pineal cellular secretory activity might also be reduced. If the latter phenomenon does occur in these circumstances, it might be taken as an indication of suppression of endogenous pineal secretion caused by increased circulating pineal hormone owing to the injection of bovine pineal extract.

The pineal glands of rats given daily intraperitoneal injections of a bovine pineal extract for 28 days starting at the age of 21 days showed decreased numbers of nucleoli and intranuclear phloxinophilic granules. This finding was taken to indicate depression of pineal cellular secretory activity owing to the increase in circulating pineal hormone caused by the injection.

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[1] Wislocki, G. B., and Dempsey, E., *Endocrinol.*, **42**, 56 (1948).
[2] Bargmann, W., "Handbuch der vergleich. Anat. Menschen", **6** (4), 309 (1943).
[3] Oksche, A., *Anat. Anz.*, **102**, 404 (1954).
[4] Caspersson, T. O., "Cell Growth and Cell Function" (W. W. Norton and Co., New York, 1950).
[5] Quay, W. B., *J. Morph.*, **98**, 71 (1956).
[6] Altschule, M. D., *New England J. Med.*, **257**, 919 (1957).
[7] Lendrum, A. C., *J. Path.*, **59**, 399 (1957).

Seromucoid Contents of Human Bile

Variations of seromucoid in pathological conditions and its clinical significance have been extensively investigated during the past few years. Greenspan[1], Winzler[2] and others have shown the seromucoid contents in the blood of normal human subjects to be $12{\cdot}4 \pm 0{\cdot}5$ mgm. per cent as expressed in hexose, and $3{\cdot}38 \pm 0{\cdot}27$ mgm. per cent as expressed in tyrosine. Seromucoid contents in the blood are significantly increased in obstructive jaundice and are decreased to very low figures in jaundice due to hepatitis. These pathological variations suggest the possibility of the liver playing an important part in seromucoid metabolism. Since for the time being the site of formation and metabolic fate of seromucoid are unknown, it has occurred to us that it might be synthesized by the liver or at least partly eliminated in the bile. We have therefore investigated the presence and contents of seromucoid in human hepatic bile.

Samples of hepatic bile draining through a Kerr tube were collected from patients who had undergone cholecystectomy. The bile was collected in the morning in post-absorptive conditions, during the last days preceding the removal of the Kerr drain, when the bile contained but a few cells and flakes of mucus were no longer present. Hepatic bile was also collected by duodenal catheterization in three patients who had undergone cholecystectomy 2, 3 and 5 years previously. The bile was immediately centrifuged at 12,000 r.p.m. for 15 min. and then filtered through Schleicher Schüll filter paper No. 589 (blue band).

Table 1. Seromucoid Contents of Human Hepatic Bile

Hepatic bile case	Hexoses (mgm. per cent)	Tyrosine (mgm. per cent)	Total solids (gm. per cent)	Hexoses in mgm. per 100 gm. of total solids	Tyrosine in mgm. per 100 gm. of total solids
P. E.	8·0	1·27	2·84	281	52·0
T. A.	6·0	0·95	2·50	240	37·8
G. O.	13·5	1·81	2·35	574	77·0
A. B.	9·2	0·38	3·08	298	12·0
T. P.	8·6	0·29	3·84	224	8·0
T. M.	2·4	1·14	4·12	58	27·0
C. V.	9·0	4·00	4·70	191	85·1
M. G.	12·0	6·80	3·37	356	101·0
Z. I.	7·0	2·64	5·56	126	47·0
W. K.	8·5	1·10	3·02	281	36·4
S. A.	6·7	0·90	2·75	244	32·6
V. H.	5·8	2·50	2·85	203	87·7
C. B.	6·5	0·85	3·20	203	27·7
±*S.E.*	7·93 ± 0·89	1·89 ± 0·49	3·39 ± 0·25	252·23 ± 18·52	48·5 ± 16·31

The clear bile obtained by these means was tested for the presence of seromucoid according to Winzler's method as modified by Weiner and Moshin[3]. Tyrosine as well as hexose contents were determined.

The total solid contents were determined in the same samples.

Seromucoid was present in all the samples of hepatic bile investigated. The seromucoid concentration in hepatic bile, as expressed in mgm. hexose and mgm. tyrosine per 100 gm. of total solids, the averages of these figures and the standard errors of the averages are listed in Table 1.

It can be seen that :

(1) The total solid contents of hepatic bile display a considerable degree of variation (3·39 ± 0·25 gm. per 100 ml.).

(2) There are great variations of seromucoid concentrations in fresh hepatic bile, both as expressed in terms of hexoses (7·93 ± 0·89 per 100 ml.) and in terms of tyrosine (1·89 ± 0·49 mgm. per 100 ml.).

(3) These fluctuations are not reduced by referring seromucoid concentrations to the total solid contents of the bile ; it follows that variations of the latter cannot wholly account for these fluctuations.

No investigations were made on hepatic bile obtained by duodenal catheterization from normal subjects in order to avoid so far as possible the contamination of hepatic bile samples by mucus secreted by the extrahepatic bile ducts and by the duodenal wall. By collecting the bile draining through a Kerr tube, this bias is minimized. We consider the presence of seromucoid in hepatic bile to be unquestionable evidence of the elimination of this glycoprotein by the liver. This might be due either to excretion through the liver of the seromucoid present in the blood, or to synthesis in the liver of the seromucoid which is afterwards eliminated via the bile and possibly the blood. Further investigations are required in order to show seromucoid synthesis in the liver. However, the presence of seromucoid in hepatic bile is clear evidence of the part played by the liver in the elimination of this substance.

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[1] Greenspan, E. M., and Dreiling, D., *Amer. Med. Assoc. Arch. Int. Med.*, **91**, 474 (1953).
[2] Winzler, R., "Methods of Biochemical Analysis", **2**, 279 (Interscience Pub., 1955).
[3] Weiner, H. E., and Moshin, J. R., *Amer. Rev. Tubercul.*, **68**, 594 (1952).

Distribution of Chromactivating Hormones in the Pituitary of the Minnow

In a previous communication[1], I have described the distribution of melanophore-aggregating hormone in the pituitary of the minnow (*Phoxinus phoxinus*). By use of the method described there the distribution of minnow erythrophore-dispersing and frog melanophore-dispersing factors in the minnow pituitary has been investigated.

Erythrophore-dispersing activity was determined by scoring the degree of reddening around the pectoral and pelvic fins of black-adapted minnows (60–65 mm. long) from 0 (no reddening) to 6 (maximum reddening) 1 hr. after intraperitoneal injection of the aqueous gland extract ; the minnows used were selected for uniformity of response to a standard dose of an extract of whole minnow pituitaries. Frog melanophore-dispersing activity was determined by measuring the melanophore-index of the web melanophores of the hind foot of white-adapted frogs (*Rana temporaria*) (20–25 gm. in weight) immediately prior to injection of the aqueous gland extract into the dorsal lymph sac and again 1 hr. after the injection. The results are summarized in Table 1.

Table 1

		Pituitary region	
		Anterior	Posterior
Response of erythrophores of black-adapted minnows. Change in erythrophore score 1 hr. after injection	No. of animals	15	15
	Mean response	1·33	4·93
	S.D.	2·225	1·982
		$P < 0.01$	
Response of melanophores of white-adapted frogs. Change in melanophore-index 1 hr. after injection	No. of animals	6	6
	Mean response	1·27	3·47
	S.D.	1·212	0·392
		$P < 0.01$	

The experiments reveal that the erythrophore-dispersing and melanophore-dispersing factors in the minnow pituitary are distributed in the same way as the melanophore-aggregating factor ; that is, they are present in both the anterior and posterior portions but in significantly higher concentration in the posterior portion. Stahl[2] has described two distinct cell types in the meta-adenohypophysis of *Mugil* and suggests that they represent the source of the melanophore-aggregating and melanophore-dispersing hormones. If this is true it is necessary to explain the presence of substantial amounts of these hormones in the anterior part of the gland. Comparison of the values determined for hormone activity in the two regions of the gland with dose-response curves (unpublished work) suggests that the level of both melanophore-aggregating and melanophore-dispersing hormone in the posterior part is about five times greater than that in the anterior part. In view of the crude technique adopted in separating the two portions of the gland it would be reasonable to suppose that some 20 per cent of the meta-adenohypophysis was in fact included in the anterior portion in these experiments. The results described here and in the previous communication thus lend support to Stahl's suggestion. It must be admitted, however, that such an interpretation cannot easily be reconciled with the

observations of Healey[3] on the minnow or Enami[4] on *Parasilurus asotus*.

The similarity in distribution of the melanophore-dispersing and erythrophore-dispersing factors adds support to the generally accepted view[5] that both effects are brought about by a single hormone. However, the results of these experiments do not preclude the possibility that either three distinct hormones, or a single hormone causing melanophore-aggregation and erythrophore-dispersion in the minnow, and melanophore-dispersion in the frog, are involved.

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[1] Kent, A. K., *Nature*, **183**, 544 (1959).
[2] Stahl, A., *C.R. Soc. Biol., Paris*, **152**, 1562 (1958).
[3] Healey, E. G., *Bull. Anim. Behav.*, **6**, 5 (1948).
[4] Enami, M., *Science*, **121**, 36 (1955).
[5] Pickford, G. E., and Atz, J. W., "The Physiology of the Pituitary Gland of Fishes" (New York Zoological Society, 1957).

Effect of Denervation on Potassium-42 Uptake by the Rat Muscle *in vitro*

Widely different reports exist concerning the changes in potassium-42 uptake by denervated muscle. Lyman[1] found an increased uptake after denervation. Fischer *et al.*[2] could show no differences in uptake between normal and denervated muscles in the rabbit. Finally, Harris and Nichols[3], who measured the uptake of potassium-42 into denervated frog muscle *in vitro*, reported, in agreement with their previous results[4], that the uptake is decreased.

Difference between the findings of Lyman[1] and Harris and Nichols[3] may be due to: (*a*) different uptake of potassium into frog and rat muscles; (*b*) different equilibration time of the isotope; or (*c*) different degrees of atrophy in the frog and rat muscles. Muscle atrophy in the frog, which was studied by Harris and Nichols[3], progresses very slowly, whereas muscles in the rat studied by Lyman[1] atrophy considerably more during the same period.

For this reason we investigated the question whether uptake of potassium-42 into frog and rat muscles is different when the equilibration time of the isotope and the degree of muscle atrophy are the same. The uptake of potassium-42 into normal and denervated diaphragm of the rat was therefore measured five days after denervation, that is, in a mammalian muscle, in which atrophy begins 20–40 days after denervation[5].

Experiments were performed on male rats of 90–100 gm. body-weight. The diaphragm was denervated by unilateral ligature of the phrenic nerve by the abdominal route. Five days after denervation, rats were killed and completeness of denervation was controlled by faradic stimulation of the phrenic nerve. The diaphragm was excised together with the rib, and after dividing it into halves the centrum tendineum was removed and the hemidiaphragms were incubated in 5 ml. of saline containing

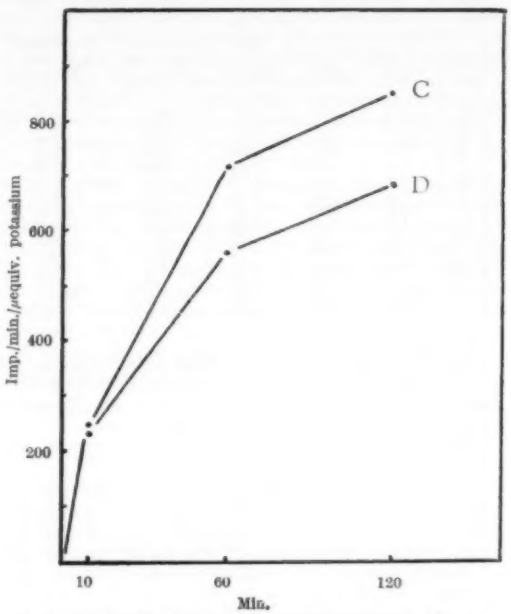

Fig. 1. Uptake of potassium-42 by normal and denervated hemidiaphragm. *D*, Denervated; *C*, control muscle

chloride labelled with potassium-42. Following incubation, the hemidiaphragms were carefully washed, isolated from the rib, and gamma-activity of the tissue was measured. The total content of potassium was determined by the flame photometer in the same way as previously described[6].

It was found that the uptake of potassium-42 by denervated hemidiaphragm is lower than in the control half (Table 1). The decrease in uptake becomes evident only after a longer period of incubation. After 10 min. incubation the uptake was the same on both the normal and denervated halves. Following 60 min. equilibration, the uptake was decreased by 118 ± 14·6 imp./min./μequiv. potassium, that is, by 16·6 per cent, while after 120 min. the uptake was decreased by 169 ± 41·5 imp./min./μequiv. potassium, that is, by 19·9 per cent (Fig. 1).

It was concluded from these results that under the same experimental conditions the uptake of potassium-42 by denervated muscle of the rat is decreased similarly to that of frog muscle[3]. Differences of uptake by denervated muscles are not caused by tissue specificity. The equilibration time of the isotope is an especially important factor when studying this uptake. The lower potassium-42 uptake

Table 1. Uptake of Potassium-42 by Normal and Denervated Halves of the Diaphragm

Time of incubation (min.)	Imp./min./μequiv. potassium		
	Denervated (*D*)	Control (*C*)	*D*—*C*
10	230	244	− 14 ± 23·8
60	596	714	− 118 ± 14·6
120	681	850	− 169 ± 41·5

Hemidiaphragms were incubated in 5 ml. Krebs–Ringer–bicarbonate saline with chloride labelled with potassium-42 (0·25 μc./ml.) in Erlenmeyer flasks at 37° C. The gaseous phase in the flask was 95 per cent oxygen + 5 per cent carbon dioxide. Each value is the average of five determinations.

occurred neither in our experiments nor in those of Harris and Nichols[3] until after a long period of equilibration. Lyman[1] showed an increased uptake after 2–9 min. of equilibration. However, in two experiments in which equilibration time lasted for 4 hr., he also found less potassium-42 in the denervated muscle (75 and 89 per cent). As has been shown in the literature as well as in the present report, the uptake of potassium-42 by denervated muscle following a longer period of equilibration is decreased. This conclusion further corroborates our previous findings concerning the restitution of potassium following various experimental conditions which were found to be retarded[7,8].

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[1] Lyman, Ch. P., *Amer. J. Physiol.*, **137**, 392 (1942).
[2] Fischer, E., Moore, J. W., Skowlund, H. V., Ryland, K. W., and Copenhaver, N. J., *Arch. Phys. Med.*, **31**, 429 (1950).
[3] Harris, E. J., and Nichols, J. G., *J. Physiol.*, **131**, 473 (1956).
[4] Nichols, J. G., *J. Physiol.*, **131**, 1 (1956).
[5] Sole, O. M., and Martin, A. W., *Amer. J. Physiol.*, **172**, 342 (1953).
[6] Drahota, Z., Klicpera, M., and Žák, R., *Biochim. Biophys. Acta*, **28**, 31 (1958).
[7] Drahota, Z., Gutmann, E., and Vrbová, G., *Physiol. Bohemoslov.*, **6**, 77 (1957).
[8] Drahota, Z., and Hudlická, O., *Physiol. Bohemoslov.*, **7**, 489 (1958).

Histochemical and Biochemical Studies of Rat Liver in Experimental Cirrhosis after Partial Hepatectomy

As an outcome of our earlier work on experimental cirrhosis[1,2] and regeneration of the liver[3] it seemed of interest to study the dynamics of the regenerative process in the normal and cirrhotic liver after partial hepatectomy.

The experiments were made on 90 male Wistar rats of 170 gm. average body-weight divided into two groups and kept on a standard diet. In the animals of group 1 cirrhosis was produced with carbon tetrachloride (0·1 ml. subcutaneously every fourth day, a total of 29 doses over a 120-day period), while group 2 was untreated. Partial hepatectomy (65–70 per cent) was made on both groups and rats were killed 24, 48, 72 and 96 hr. after surgery. The removed lobes and postsurgical remnants were examined by histomorphological, histochemical and biochemical methods.

Succinic dehydrogenase activity (neotetrazolium method) in the cirrhotic liver proved to be somewhat less than in the normal one. The activity of this enzyme decreased very much in both groups 24 hr. after partial hepatectomy. This decrease in normal livers persisted unaltered for 3 days and a partial return of the activity was seen only on the fourth day. In contrast to this, the activity of succinic dehydrogenase of the regenerating cirrhotic liver reached that of the intact (unoperated) cirrhotic liver within 48 hr., and after 96 hr. closely approached that of the normal controls. The succinic dehydrogenase activity was also measured by a quantitative biochemical procedure[4]; the results of these determinations confirmed entirely those obtained by the histochemical technique.

Acid and alkaline phosphatase activities were studied both by histochemical procedures (Gomori–Moffat) and by quantitative methods using liver suspensions[5,6]. The results of both types of analysis were in agreement; the quantitative results are shown in Table 1. There was a substantial increase in the alkaline phosphatase activity both in the cirrhotic and normal livers 24 and 48 hr. after partial hepatectomy, the activities returning to the pre-operative levels by 96 hr.; the alkaline phosphatase activities in cirrhotic livers were generally much higher than in the normal organ. The acid phosphatase-levels were somewhat variable in normal livers after hepatectomy, but showed substantial increases in the regenerating cirrhotic livers.

Table 1. Acid and Alkaline Phosphatase Activities in Normal and Cirrhotic Liver after Partial Hepatectomy

Hours after hepatectomy	Normal liver			
	Acid phosphatase		Alkaline phosphatase	
	Per wet weight	Per dry weight	Per wet weight	Per dry weight
0	500	1,530	43	130
24	323	1,075	106	351
48	412	1,425	187	646
72	614	2,106	122	418
96	497	1,651	38	124
	Cirrhotic liver			
0	295	1,143	84	324
24	416	1,445	206	717
48	577	2,042	360	1,274
72	426	1,508	100	354
96	515	1,840	116	378

The activities are given as μgm. inorganic phosphorus liberated per hr. from β-glycerophosphate by 100 mgm. of wet or dry liver tissue. The inorganic phosphorus was determined by the method of Fiske and Subbarow (ref. 5).

In histochemical preparations the activity of lipase (Gomori's method) was seen to decrease early after partial hepatectomy in both groups of animals, but a return to normal followed quickly.

Cytochrome oxidase was also measured according to the method of Mitolo and Ruccia[7]. The activity of this enzyme showed a periodic change (decrease→return to normal→decrease) in the regenerating normal liver, whereas in the regenerating cirrhotic liver there was more than a threefold increase even on the fourth day after hepatectomy.

We could not observe any change in the architecture of the cirrhotic livers after partial hepatectomy. The mitotic activity seemed, however, greater in the cirrhotic post-operative remnants than in the non-cirrhotic ones during the early days of regeneration.

The results indicate that the enzymic responses of the cirrhotic liver after partial hepatectomy are more intense than those of the normal regenerating one. A more detailed study of this phenomenon and the evaluation of its significance in the regenerative process is in progress.

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[1] Korpássy, B., *Cancer Res.*, **19**, 501 (1959).
[2] Korpássy, B., and Kovács, K., *Brit. J. Exp. Path.*, **30**, 266 (1949).
[3] Horváth, E., and Kovács, K., *Z. ges. exp. Med.*, **127**, 236 (1956).
[4] Domján, Gy., and Kovács, E., *Enzymologia* (in the press).
[5] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, **66**, 375 (1925).
[6] Flexner, L. B., and Flexner, J. B., *J. Cell. Comp. Physiol.*, **31**, 311 (1948).
[7] Mitolo, M. E., and Ruccia, D., *Arch. Sci. Biol.*, **39**, 132 (1955).

RADIOBIOLOGY

Whole-Body Turnover of Selenium in the Rat

Selenium has recently been shown to be an essential micronutrient for rats[1] and ruminants[2]. These discoveries have directed attention to the lack of information concerning the metabolism of selenium in subtoxic amounts. The present communication gives results for whole-body turnover, or total excretion, of selenium by the rat over a prolonged period of time.

Hooded rats of the Long–Evans strain were used. They were maintained on a complete stock colony ration (*ad libitum*) and weighed approximately 150 gm. at the time of administration of radioactive selenium; 0·0011 mc. of selenium-75 in physiological saline was administered interperitoneally. The total selenium content of the dose was 0·14 mgm. and the selenium was present as sodium selenite. The total selenium-75 content of the rats was determined three times daily for the first two days, twice daily for one week and daily for a second week. For measurement, individual rats were placed in a ventilated stainless steel tube and confined to a length approximately equal to that of the rat's body. The rat was then suspended approximately 20 cm. above a scintillation counter for measurement of radiation. The counter used was a standard well-counter operated without the top lead shield. The entire top surface of the crystal was unshielded. The 0·269-MeV. gamma-ray of selenium-75 was counted, using a single-channel pulse-height analyser and scaler. For calibration, a vial containing an aliquot of the dose was substituted for the rat. The standard error of measurement was 2·3 per cent as determined from duplicate counts[3]. There was no difference in the error of measurement between two operators.

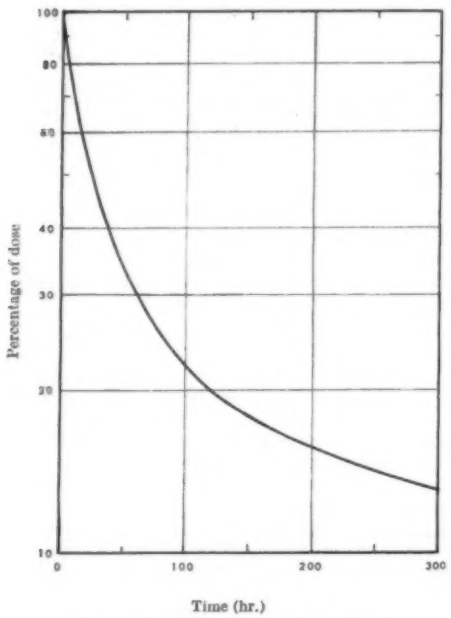

Fig. 1. Radioselenium turnover in the rat

Table 1. Whole-body Turnover of Selenium in the Rat

Rat No.	Rate constants (hr.$^{-1}$): K_1	K_2
0	0·037	0·0025
1	0·037	0·0018
2	0·036	0·0021
3	0·037	0·0025
6	0·040	0·0023
7	0·036	0·0021
9	0·038	0·0021
Mean	0·037	0·0022
Standard deviation	0·001	0·0002
$t_{1/2}$ (hr.)	19	318

The results were analysed by considering the whole-body turnover to be describable by a set of consecutive first-order chemical reactions. The rate constants for the various segments of the turnover curve were computed, fitting the data by the method of least squares.

For the first 300 hr. after injection of selenium-75, the whole-body turnover may be described by two first-order processes of widely differing rate constants (K_1 and K_2). A graph presenting the average results is shown in Fig. 1. The initial slope of the curve is determined by K_1 and the final slope by K_2. The individual rate constants are presented in Table 1.

Previously published work of selenium metabolism was for periods of less than 50 hr. and hence comparison is difficult. Also, other studies measured excretion, rather than retention, and often ignore one of the three routes of selenium excretion—urine, fæces and expired air. Harshfield[4] found the 'total recoverable selenium' in the rat to be about 75 per cent of dose at 6 hr. after injection and about 65 per cent of dose at 12 hr. These results agree with the present work. Harshfield found, however, no decrease in 'total recoverable selenium' between 12 and 48 hr. post-injection as was found here (see Fig. 1). McConnell[5] found the urine and fæcal excretion of selenium-75 from the mouse to be about 45 per cent of dose in the first 24 hr. The present work indicates a total excretion of about 48 per cent of dose in an equivalent time. Heinrich and Kelsey[6] reported the 'total recoverable selenium' in the mouse to be only 16·5 per cent of dose at 48 hr. after injection—considerably less than that found here for the rat.

The presence of two rate constants governing selenium turnover indicates that selenium is involved in the metabolism of the animal. Selenium is transferred from one (rapidly excreted) form to another (slowly excreted) form. Two choices are available: (1) the rate-limiting processes are the renal clearance of two chemically different forms of selenium; and (2) the rate-limiting process for the smaller rate constant is the rate of release of selenium from tissues in which it is fixed.

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[1] Schwarz, K., and Foltz, C. M., *J. Agric. Chem. Soc.*, **79**, 3292 (1957).
[2] Muth, O. H., Oldfield, J. E., Remmert, L. F., and Schubert, J. R., *Science*, **128**, 1090 (1958).
[3] Youden, W. J., "Statistical Methods for Chemists", **16** (Wiley, New York, 1951).
[4] Harshfield, R. D., "A Study on the Metabolism of Selenium^{75} in the Mouse", M.A. thesis, University of South Dakota (1953).
[5] McConnell, K. P., *J. Biol. Chem.*, **141**, 427 (1941).
[6] Heinrich, M., and Kelsey, F. E., *J. Pharmacol. Exp. Therap.*, **114**, 28 (1953).

Selection of Iodinated Protein in the Young Mouse

THE suckling mouse absorbs antibody readily when whole immune serum is administered by mouth[1] and transmission to the circulation is selective in the sense that antibodies prepared in a variety of species are characterized by different concentration quotients in the recipient plasma[2]. During the course of the work with serum protein preparations labelled with iodine-131 in other species a criterion of the physiological integrity of the labelled material was sought which would be sensitive and readily determined. Recourse was therefore made to gut transmission in the young mouse.

The mice employed throughout the work were L.A.B. Greys, obtained by courtesy of Dr. Lane-Petter in 1955 and bred randomly since then. Preliminary experiments suggested the standard conditions of testing. Protein, labelled by the jet-burette method[3], was made up finally in 1 per cent solution. This was fed to 8-day old mice which were killed 3 hr. later, the blood collected, and the activity of the serum protein determined after tungstic acid precipitation.

Values are expressed as the ratio :

$$\text{concentration quotient} = \frac{\text{c.min.}^{-1}\,\text{ml.}^{-1}\ \text{serum protein}}{\text{c.min.}^{-1}\,\text{ml.}^{-1}\ \text{preparation fed}}$$

Fig. 1*A* presents the regressions of the concentration-quotient with dosage for two levels of iodination of aliquots of a frozen-dried stock preparation of rabbit globulin which was prepared by final precipitation from 12 per cent sodium sulphate[4]. It is apparent that a very sharp selection occurs between these two preparations, although they differ only in the level of iodine-127 per molecule of globulin.

Fig. 1*B* illustrates the relation of concentration quotient to iodination-level at a chosen dose of 0·03 ml. fed, and is thus in effect a calibration curve for the testing of subsequent preparations of this protein

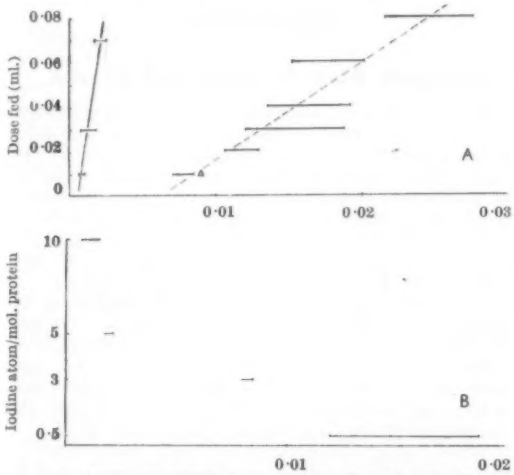

Fig. 1. Absorption of 1 per cent solutions of rabbit globulin from the gut of the 8-day old mouse. The means ± 2 standard error of groups of six mice are plotted; within each relationship each group was composed of one sibling of each of six litters. (*A*) Dosage relation : – – – –, regression where the mean iodination-level is 0·5 atom/mol. $\delta = 4{\cdot}4$; ———, regression where the level is 10 atom/mol. $\delta = 49{\cdot}4$; △ = mean concentration quotient of rabbit anti-sheep red cell serum from ref. 2. (*B*) The test calibration curve : dose fed 0·03 ml.

at any future date. It is clear that there is no significant difference between the concentration quotient at the mean iodination-level of 0·5 atom per molecule and that for antibody from whole rabbit serum at the same dosage (Fig. 1*A*), so that it is possible to take the concentration quotient of isotope at this level as being maximal for the stock preparation of globulin in these experimental conditions. Since the reduction of concentration quotient at the mean level of 3 atoms per molecule is already highly significant, the proposed test is clearly a sensitive one. It is thus possible, by feeding small groups of mice, to check the integrity of labile preparations in the course of a few hours.

Selection between molecules differing only in the number, and presumably the distribution, of labelled tyrosine residues, since it is so sharp, may suggest that some at least of the tyrosine residues of the molecular surface normally play a part in selection of the protein by the entoderm cell.

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[1] Morris, I. G., *Proc. Roy. Soc.*, B, **148**, 84 (1957).
[2] Hemmings, W. A., and Morris, I. G., *Proc. Roy. Soc.*, B, **150**, 403 (1959).
[3] McFarlane, A. S., *Biochem. J.*, **62**, 135 (1956).
[4] Kekwick, R. A., *Biochem. J.*, **34**, 1248 (1940).

Cæsium-137 in Toronto Milk during 1959

MEASUREMENTS of the changing cæsium-137 content of milk have been carried out in many parts of the world, particularly in the United States[1] and the United Kingdom[2]. The Los Alamos group measured the activity of Ontario milk during 1958[3]. Here we present results of measurements on milk taken from the Toronto milk-shed during the period April 1959–February 1960.

The measurements were carried out using as gamma-ray detector a 5-in. sodium iodide crystal placed in the University of Toronto 'steel room'[4]. 670 gm. of dried skimmed milk, received within a few days of production, were placed in a thin copper vessel immediately below the crystal. Counting was normally for 75 min.

The only photo-peaks observed were at energies of 0·66 MeV. and 1·46 MeV., corresponding to cæsium-137 and potassium-40 activities. By comparison of the areas of these photo-peaks, and making suitable corrections[4], the ratio cæsium-137/potassium of a sample is obtained. Calibration of the apparatus was carried out regularly using a weighed amount of potassium chloride in a standard copper vessel, and thus the potassium content of the milk could be determined, corrections once again being necessary for the differential absorption of the potassium gamma-rays in the potassium chloride and in the dried milk. It was in fact found that the potassium content of each sample of dried milk was approximately the same, the average value being 3·7 mgm. of potassium per gm. of dried milk.

The statistical error associated with the measurement of the potassium content of any one particular milk sample did not exceed 2 per cent.

The results for cæsium content during the period of observation, expressed in cæsium units (C.U.), that is, μμc. cæsium-137 per gm. of natural potassium, are given in the lower part of Fig. 1. For comparison,

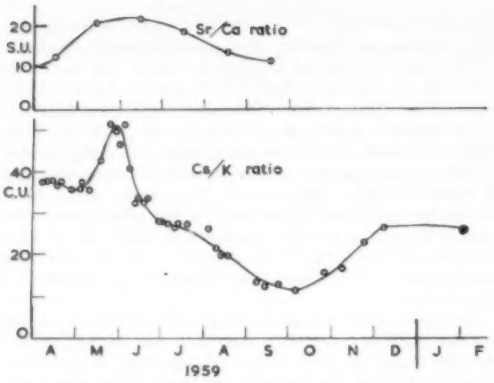

Fig. 1. Lower graph: change with time of the cæsium content of Toronto milk during April 1959–February 1960. (c.u. = μμc. cæsium-137 per gm. potassium.) Upper graph: Canadian average strontium-90/calcium ratio in milk (ref. 5). s.u. = μμc. strontium-90 per gm. calcium

the averaged ratio strontium-90/calcium for milk in Canada for the corresponding period[5] is given in the upper part of Fig. 1.

We interpret the cæsium curve in the following way:

(1) During April, cows are in barns, on winter feed, the cæsium-137 content of which will reflect the mean rate of fallout of the previous summer. Thus little change in the ratio cæsium/potassium in milk from these cows is expected in this period.

(2) The cows from which our milk came went out to pasture during the second week of May. This pasture would be contaminated with the fallout from the low-altitude Russian bomb tests of late 1958. As a result, the cæsium content of the milk rose rapidly after the change over to summer-feed. It will be noted that the time for the cæsium content to change by half the difference between the winter-level and the peak is approximately 12 days. The biological half-life of cæsium in the cow is stated to be about 20 days[6].

(3) The Russian fallout was apparently retained only a short time in the atmosphere, and after the rapid rise to the peak of 51 c.u. at the end of May, the cæsium activity in milk fell to the low value of 11·6 c.u. in October. This decrease seems to have two components, one rapid and one relatively slow. We attribute the former to the fact that cows grazing on new grass would pick up activity collected on the grass over about one month, while the second-growth grass would only have on it fallout collected over perhaps one or two weeks.

(4) In early November or late October the cows returned to the barns, to feed on hay, etc., cut during the early summer of 1959. As a result, the ratio cæsium/potassium rose, this time to a level equal to that of July 1959. Unfortunately, supplies of dried milk have been poor during the winter, and the presence of a plateau is only shown by the approximate equality of runs in late November and early December using dried milk, and one run in February 1960 using liquid milk.

It may be noted that during the summer decrease, during July 1–September 30, the cæsium content of milk fell by a factor of 2. On the assumption that cæsium-137 intake by cows is via direct fallout on foliage and not via the roots of plants, this suggests a half-life in the troposphere of the fallout of the order of 2 months.

The fact that there are two distinct rates of decrease of the cæsium/potassium ratio supports the assumption that most of the cæsium entering cattle comes directly from the foliage rather than via the grass root-system.

Comparison with the strontium-90 results show that the rise occurs in strontium content at the same time as the rise in cæsium content, and that correspondingly there is a fall throughout the summer in the strontium activity. However, the ratios of the spring peak values to the winter 1958–59 plateau values for cæsium-137 and strontium-90 are different (10 : 7 and 2 : 1 respectively) and the rate of decrease of the cæsium-137 content of milk was greater than that for strontium-90.

The October 1959 low value of 11·6 c.u. corresponds to an activity of approximately 19 μμc. of cæsium-137 per (Imperial) quart of milk. Assuming a 115-day biological half-life for cæsium in the body[7], and an intake of one quart of milk a day, this activity leads to a total maximum body burden due to this source of cæsium of $3{\cdot}1 \times 10^3$ μμc., which is 10,000 times less than the maximum permitted amount in the body[8].

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[1] Langham, W. H., and Anderson, E. C., *Health Physics*, **2**, 30 (1959).
[2] Booker, D. V., *Nature*, **183**, 921 (1959). Anderson, W., Burton, L. K., and Crookall, J. O., *ibid.*, **184**, 89 (1959).
[3] Anderson, E. C., Clinton, B., and Shields, J. A., Los Alamos Report 2296 (1959).
[4] McNeill, K. G., and Green, R. M., *Can. J. Phys.*, **37**, 683 (1959).
[5] Department of National Health and Welfare Quarterly Reports on Levels of Sr 90 in Canadian Milk Powder Samples, June, September and December 1959.
[6] Hood, S. L., and Comar, C. L., *ORO*-91 (1953).
[7] McNeill, K. G., and Green, R. M., *Can. J. Phys.*, **37**, 528 (1959).
[8] National Committee on Radiation Protections and Measurement, NBS Handbook, 69 (1959).

HISTOLOGY

A Specific Stain for Mast Cell Granules

Examination of tissues stained for mast cells with a variety of accepted techniques has shown a need for a more specific staining method. The use of the conventional metachromatic dyes, though resulting in satisfactory preparations in many cases, presented problems of identification of mast cell granules in some tissues, for example, in lymph nodes where the intense nuclear orthochromatic staining of surrounding cells obscures metachromatic granules.

As recently as 1957, Barlow[1] was prompted by "a lack of a sufficiently reliable and selective method of identification" for mast cells to formulate a tribasic stain for their differential staining. The constituents for this tribasic stain were acridine red, neutral red, and basic fuchsin, but unfortunately the staining technique was lengthy and complicated.

When using metachromatic dyes, background or orthochromatic staining of tissue elements can be completely suppressed by acidifying the dye solution and adding metallic cations in suitable concentrations[2]. In dilute solutions, however, lengthy periods of immersion in the dye were necessary to produce even faint metachromatic staining of the mast cell granules. In order to increase the speed and intensity of the granule staining, dye concentrations

were increased, and background staining was avoided by increasing the metallic cation concentrations.

The tissues employed were obtained from the lip and tongue of young adult Chester Beatty albino rats. These tissues were fixed in 10 per cent neutral formol saline, embedded in paraffin wax–'Celloidin' by Peterfi's method, and cut in serial section at 7μ thickness. These techniques have been shown to be satisfactory for the demonstration of mast cells in tissues obtained from the albino rat[3].

A 0·01 per cent toluidine blue solution buffered at *p*H 3·0 with a 1 in 10 dilution of McIlvaine's sodium hydrogen phosphate citric acid buffer was found to stain satisfactorily mast cell granules in 10 min. A concentration of 2 gm. per cent crystalline magnesium chloride, that is, 0·2 *N* concentration, was required to prevent background or orthochromatic staining. Except for faint staining of cartilage matrix, this solution was specific for the granules of mast cells (Fig. 1).

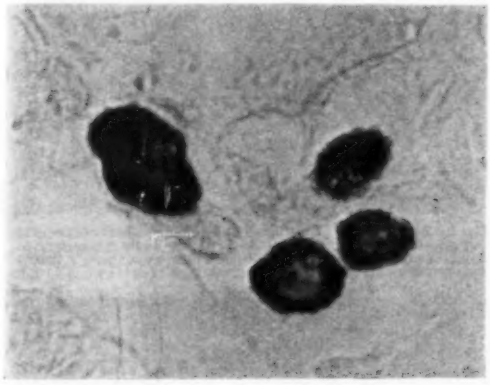

Fig. 1. Staining of mast cell granules with complete suppression of background staining. (× 1,000)

Nuclear fast red stained the nuclear material without interfering with the granule staining.

W. Montagna (personal communication, 1956) suggested that much 'washing out' of a metachromatic dye by the low-grade alcohols during dehydration procedures could be avoided by using tertiary butyl alcohol instead of the various grades of ethyl alcohol. As tertiary butyl alcohol was unobtainable, the closely related *iso*-propyl alcohol was used as a substitute and found to have no apparent deleterious effect on the toluidine blue staining.

Montagna's recommendation to employ toluene as a clearing agent has also given satisfactory results. In later work terpineol has been substituted for toluene, as it is less volatile and, therefore, easier to use. The results were similar to those obtained with toluene.

Diffusion of the dye into the mounting medium has been prevented by employing toluene-balsam, and avoiding heat in drying the mountant. Sections prepared in this manner have remained unchanged over a period of three years.

The technique for the 'specific' stain is as follows: (1) remove paraffin wax with xylene, and hydrate in descending grades of ethyl alcohol; (2) wash in distilled water; (3) immerse in a 'pretreatment' solution of a 1 in 10 dilution of McIlvaine's sodium hydrogen phosphate citric acid buffer at *p*H 3·0 for 1 hr.; (4) stain for 10 min. in a 0·01 per cent toluidine blue (Gurr 2706) solution in a 1 in 10 dilution of buffer at *p*H 3·0 to which 2 gm. per cent crystalline magnesium chloride have been added; (5) counterstain with 0·1 per cent nuclear fast red (Gurr 3569) for 5 min.; (6) rinse in distilled water; (7) dehydrate with three changes of *iso*propyl alcohol for 2 min. each; (8) clear in toluene or terpineol; (9) mount in toluene-balsam, and dry away from heat.

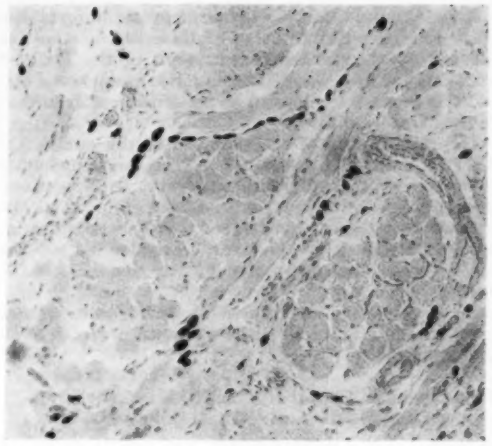

Fig. 2. 'Specific' stain preparation with mast cells clearly visible. (× 120)

With this technique the granules of mast cells are stained heavily with the toluidine blue, and the cartilage matrix faintly blue. Nuclei are stained red (Fig. 2).

In order to establish the precise relationship of the nuclei to the granules of the mast cells stained by the 'specific' stain, a composite Feulgen reaction 'specific' stain was carried out on sections of lip and tongue.

The Feulgen reaction (after Pearse[4]) was carried out first, without counterstain. The sections were then immersed in the 'pretreatment' buffer solution for 1 hr., and then stained with the 'specific' stain. Nuclear fast red counterstain was omitted from the 'specific' stain procedure.

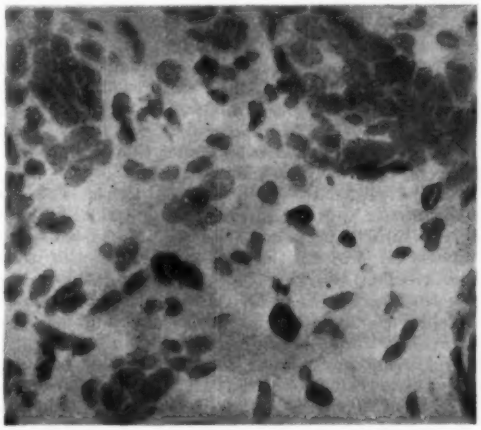

Fig. 3. Composite Feulgen reaction–'specific' stain. (× 540)

It was found that the subsequent staining of the mast cell granules by the toluidine blue was not affected by the preceding treatment of the Feulgen reaction. The granules remained metachromatic, and the nuclear chromatin was stained red from the Feulgen reaction.

The relationship of the granules to the nuclei was clearly apparent in those cells which contained only moderate numbers of granules (Fig. 3). In some cases, however, the granules were so numerous that the nuclear staining was obliterated. The distribution of the Feulgen-positive material corresponded to the nuclei demonstrated by the nuclear fast red in the 'specific' stain.

The 'specific' stain applied to frozen sections has given similar results to those of paraffin wax–'Celloidin' sections.

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[1] Barlow, R. M., *J. Path. Bact.*, **73**, 272 (1957).
[2] Radden, B. G., submitted to *J. Histochem. Cytochem.*
[3] Radden, B. G., thesis accepted by the University of London for the degree of Ph.D. (1959).
[4] Pearse, A. G. E., "Histochemistry. Theoretical and Applied", 1st ed. (Little Brown and Co., Boston, 1953).

Ultrastructure of the Clear Cells in Renal Carcinomas and its Importance for the Demonstration of their Renal Origin

Most pathologists will agree that the histogenesis of the so-called Grawitz tumours of the kidney was settled by the classical paper of Stoerk published in 1908 [1]; but the arguments of this author, convincing as they were in disproving the suprarenal nature of the 'hypernephroma', were much less so in demonstrating their renal origin. That is the reason why this latter opinion has been challenged time and again. New theories have been put forward, among which those of Apitz[2] and of Feyrter[3] have attracted some attention lately.

It seemed interesting then to inquire whether the ultrastructure of the clear cells might reveal some details which would provide conclusive evidence of their renal origin.

This is indeed the case, and the main cytological feature revealed by the study of 8 clear cell carcinomas of the human kidney will be briefly outlined here.

In the electron microscope the tumour cells appear clearly delimited, separated from the vascular stroma by a typical basal membrane, identical to the similar structure in the normal kidney tubule. But whereas in the optical microscope the cytoplasm of the clear cells appears empty, here it is filled with structural elements among which three are most conspicuous: mitochondria, heavy osmiophilic fat deposits and glycogen. The abundance of mitochondria is one of the main characters of these tumour cells. Many of them appear literally stuffed with these organelles and the presence of numerous microbodies indicates that an active multiplication of the chondrioma is taking place. In all probability the high amount of fatty material noticed long ago in the clear cells is related with this extraordinary aptitude of the clear cell to produce mitochondria. This fact has to be kept in mind because it is in itself a characteristic property of convoluted tubule cells.

Fig. 1. Basal portion of a tumour cell showing the characteristic features of a convoluted tubule cell, especially smooth cytomembrane (arrows) inserted on the basal membrane (*bm*) (× 6,860)

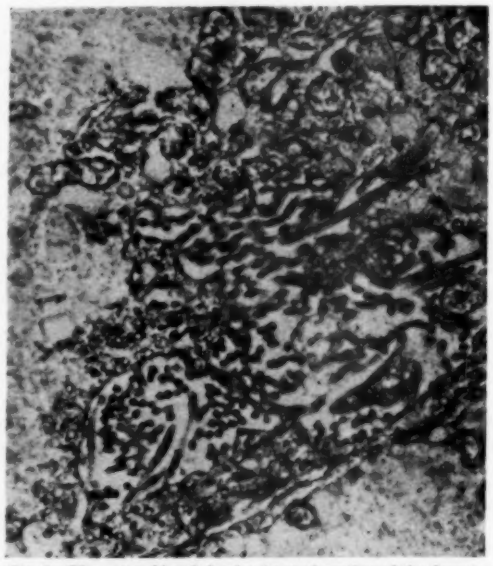

Fig. 2. Elements of brush border (central portion of the figure) belonging to a group of tumour cells (× 13,700)

But other and far more significant morphological details have come to light during these studies. In the first place the presence of smooth cytomembranes must be noticed. They form parallel sheets (Fig. 1) inserted perpendicularly upon the basal membrane. These formations exist in all cells where active exchange of liquid takes place such as in the choroid

plexus, the ciliary epithelium and the submaxillary salivary gland[4]. They are also a very prominent constituent of the convoluted tubule cells. Furthermore, a number of the tumour cells exhibit a free apical surface provided with microvilli (Fig. 2) which are identical to the embryonic form of brush borders.

Here then are ultrastructural constituents of the clear cells in renal carcinomas which indicate clearly that these tumours arise from the epithelial cells of the convoluted tubule.

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[1] Stoerk, O., *Beitr. Anat. und Allg. Path.*, **43**, 393 (1908).
[2] Apitz, K., *Virch. Arch.*, **311**, 285 (1943).
[3] Feyrter, F., "Über diffuse endokrine epitheliale Organe" (J. A. Barth, Leipzig, 1938).
[4] Pease, D. C., *J. Biophys. Biochem. Cytol.*, **2** (Supp.), 203 (1956).

BIOLOGY

Collagenase Maceration of Kidney for Microdissection

THE traditional method of preparing kidney for microdissection is by maceration of formalin-fixed tissue with concentrated hydrochloric acid[1]. This method gives an excellent preparation of loosened nephrons and collecting tubules that can be disentangled and studied by microdissection. However, it suffers from the disadvantage that the acid treatment changes the nuclei so that they cannot be demonstrated in dissected preparations by the usual staining methods. A method of maceration that would allow nuclear staining after dissection would considerably widen the scope of the technique.

A variety of alternative macerating agents has been tried without success[2]. The present communication records the successful use of the collagenase of *Clostridium histolyticum* for maceration, permitting subsequent nuclear staining, of alcohol–acetic acid-fixed kidney.

In the course of the investigation, collagenase has been tested on fresh unfixed tissue and on tissue fixed with either alcohol, alcohol–acetic acid, acetone, acetone–acetic acid, saturated mercuric chloride, saturated picric acid, mercuric-dichromate (Zenker stock) or formol saline. Phosphate and borate buffers have been compared as diluents for collagenase; formol saline and 1 per cent acetic acid have each been tried as final fixatives for the macerated tissue; and the pretreatment of tissue slices with 6 *M* urea[3] has been tested. It has been confirmed that it is not possible to use collagenase for formalin-fixed tissue. Formalin alters the collagen substrate so that it is no longer susceptible to collagenase[4,5]. Alcohol-fixation does not inhibit the activity of collagenase, but the nephrons and collecting tubules are too brittle for dissection although good nuclear staining is possible. Acetone-fixation does not inhibit collagenase, the nephrons and collecting tubules are much easier to dissect and nuclear staining is not impaired. The addition of 25 per cent glacial acetic acid to either alcohol or acetone for fixation further improves the macerated tissue for microdissection.

Slices of alcohol–acetic acid-fixed kidney[6], about 1 mm. thick, are washed for 4 hr. in running water and are then immersed in phosphate buffer at *p*H 7 containing 0·25 mgm./ml. collagenase (Collagenase *ABC* Form II obtained from Agricultural Biochemicals Corporation, New York) and 0·1 mgm./ml. chloramphenicol. After 24 hr. at 37° C. the macerated tissue is transferred to formol saline. After microdissection, portions of nephron or collecting tubule that require staining are mounted on a slide under a coverslip and stained by drawing Mayer's hæmatoxylin through with blotting paper. After a few minutes the stain is rinsed away with Scott's tapwater and the nuclei are clearly stained in pale cytoplasm.

Although collagenase-macerated material is not so easy to dissect as acid-macerated tissue, the method, by allowing nuclear staining, yields valuable information not otherwise obtainable.

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[1] Oliver, J., MacDowell, M., and Tracy, A., *J. Clin. Invest.*, **30**, 1307 (1951).
[2] Darmady, E. M., and Stranack, F., *Brit. Med. Bull.*, **13**, 21 (1957).
[3] Neuman, R. E., and Tytell, A. A., *Proc. Soc. Exp. Biol. N.Y.*, **73**, 409 (1950).
[4] Robb-Smith, A. H. T., "Nature and Structure of Collagen", edit. by Randall, J. T., 21 (Butterworths, London, 1953).
[5] Keech, M. K., *J. Path. Bact.*, **77**, 351 (1959).
[6] Clarke, J. L., *Phil. Trans. Roy. Soc.*, B, **141**, 607 (1851).

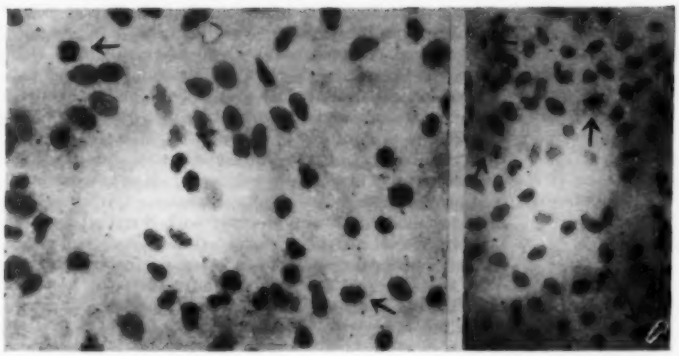
Culture of Human Leucocytes on the Chorio-allantoic Membrane of the Chick

A NEW technique has been developed for the culture of human leucocytes and other cells.

Suspensions of cells of the 'buffy coat' of heparinized human blood were introduced under sterile conditions into diffusion chambers consisting of two sheets of membrane filter ('Oxoid'), 2 cm. in diameter, cemented to a polystyrene ring. The inoculated chamber was sealed and dropped on to the chorio-allantoic membrane of the seven-day chick embryo through a slot in the egg-shell. Eggs were incubated at about 39° C. Cultures were examined at intervals to determine the state of the cells. After removal from the egg the filter disks were cut free from the polystyrene ring, placed on slides and stained with acetic orcein.

After two days of incubation many macrophage-like cells were present on the inside of both upper and lower disks of the chamber, but primarily on the upper disk. The fluid in the chamber contained mostly small lymphocytes and polymorphonuclear leucocytes which were also found on the bottom disk but apparently not attached. Present on the bottom disk was also a considerable number of large mononuclear cells which appeared to be attached to the membrane filter.

Cell divisions were first observed after four or five days of incubation and only in cells which seemed to be of the large mononuclear class. Divisions were observed in isolated cells (Fig. 1*a*) as well as in cells which were part of a group. Dividing cells were found only on the inside of the bottom disk of the chamber, that is, on the membrane filter which was in direct contact with the chorio-allantoic membrane of the chick. At five days most of the cells on the upper disk of the chamber were dead while the fluid in the chamber still contained healthy looking small lymphocytes and polymorphonuclear leucocytes. No human erythrocytes were left by this time. Chick cells were effectively excluded from the inside of the chamber.



a *b*

Fig. 1. Human leucocyte cultures. Arrows point to dividing cells. *a*, 7 days old; *b*, 14 days old

By seven days the number of cells in division was substantially increased and the cells were, for the most part, assembled into a nearly continuous sheet, epithelioid in character.

At five, seven or eight days, chambers were transferred to a new host and examined five or six days later (twelve to fourteen days from the start of the cultures). At this time the only cell-type remaining was a large epithelioid-like mononuclear cell found exclusively on the bottom disk of the chamber; this cell was several times larger than uncultured blood monocytes. All small lymphocytes and polymorphonuclear leucocytes had disappeared from both fluid and disks of the chamber and no debris was present. The frequency of divisions was higher than before (Fig. 1*b*), that is, of the order of 2 per cent.

Human foreskin fibroblasts and some embryo chick cells, cultured by the method reported above, also have yielded promising results. The technique may prove particularly useful for culturing cells which are ordinarily refractory to primary *in vitro* cultivation.

This work, part of a programme supported by the U.S. National Institutes of Health (*RG*. 5804), was done while I was Special Research Fellow (*CF*4028–*C*2) of the U.S. National Cancer Institute, on leave from the Department of Biology, Massachusetts Institute of Technology.

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Glasgow. Dec. 24.

Decomposition of Humic Acid by Fungi

Although there have been a number of publications dealing with the utilization of humic acid by microorganisms, these papers have, at the most, reported that some humic acid preparations may serve as a source of nitrogen[1,2]. No convincing evidence appears to have been published concerning the destruction or utilization of the non-nitrogenous portion of the humic acid.

As the result of a prolonged series of percolation experiments, we have isolated a number of organisms capable of decomposing humic acid. Of these the two most active are fungi and have been provisionally referred to the genera *Spicaria* and *Polystictus*. After successful tests had been completed with the presumed *Polystictus*, the Basidiomycetes, *Polystictus versicolor*, *Hypholoma fasciculare* and *Trametes rubescens*, were also tested and found to decompose the humic acid. We have previously[3] discussed the difficulties associated with investigations concerned with the microbiological utilization of humic acid, but in the work reported here it is considered that there is evidence of a genuine biological decomposition of the humic acid molecule.

The humic acid used was extracted from the B_1 horizon of a podzol soil from Delamere Forest, Cheshire, by means of 50 per cent v/v lactic acid. The extract was passed through an ion exchange column, precipitated with hydrochloric acid and then purified by dialysing, first against dilute hydrochloric acid and then distilled water. The humic acid was then dried *in vacuo* at 45° C. An analysis gave the following percentage composition: carbon 50, hydrogen 3·9, oxygen 38, nitrogen 0·4, methoxyl 1 and ash 1·5. The basic medium used throughout was the one on which the effect was first observed and may not be optimal. It contained dihydrogen potassium phosphate, 0·4; dipotassium hydrogen phosphate, 0·6; magnesium sulphate, 0·5; potassium chloride, 0·5; sucrose, 20 gm. per litre, with trace elements and Fries's vitamin solution added. 200 μgm. per litre of vitamin B_{12} was also included.

Growth of the active fungi on agar containing sufficient humic acid (0·12 per cent) to produce a dark brown medium gave a clear zone associated with the fungal mycelium. There was no evidence of any accumulation of the humic acid by the mycelium as had been observed previously[3] with fungi such as *Penicillium* spp. In Petri-dish culture complete clearing of the medium occurred with *Polystictus* in four weeks.

Quantitative studies have been carried out using soil perfusion columns and large-scale cultures in penicillin flasks. At the end of the experiment the humic acid remaining in the perfusate or medium respectively was recovered. In addition to this the soil or mycelium in each case was treated with warm 1 per cent potassium phosphate solution to remove all absorbed humic acid, since it is known that not only fungal mycelium[3] but also soil colloids[4] will absorb considerable quantities of humic acid. The two extracts were combined and the humic acid precipitated and removed for final purification, drying and weighing.

The soil percolation columns contained sterile soil and were inoculated with either a spore suspension of *Spicaria* or a mycelial suspension of *Polystictus*. The results of the recovery of humic acid after a period of eight weeks are given in Table 1 and are compared with a similarly treated uninoculated control column. It can be seen that a considerable loss of the humic acid has occurred in comparison with the control.

The penicillin flasks contained 500 ml. of medium, but with the addition of 0·25 per cent agar and 0·039 per cent humic acid. They were inoculated and incubated at 25° C. for six weeks. The loss of humic acid is shown in Table 2.

Table 1. Recovery of Humic Acid from Soil Percolation Columns after Eight Weeks

	Humic acid (mgm.)		Loss (per cent)
	Added	Extracted	
Control	81	77	4·9
Spicaria	66	44	33·3
Polystictus	77	44	42·8

Table 2. Recovery of Humic Acid from Penicillin Flask Cultures after Eight Weeks

	Humic acid (mgm.)	
	Added	Recovered
Control	195	210
Spicaria	195	162
Polystictus	195	70

The possibility was considered that the humic acid may have been reduced to a leuco form which was not precipitated by acids. Tests showed that this was not so and in any event the strong oxidase activity of *Polystictus*[5] makes this possibility very remote.

The ability of Basidiomycetes to decolorize humic acid suggests a similarity to the bleaching effect on malt agar reported by Nobles[6] and Cartwright and Findlay[7]. The colour of malt agar is due to the caramelized carbohydrates and is therefore akin to saccharo-humic acid. Tests with saccaro-humic acid prepared from sucrose with hydrochloric acid have shown that this is also decomposed by *Spicaria* and *Polystictus*. However, this does not necessarily mean similarity in structure between the two humic acids in view of the ability of *Polystictus versicolor*[8] to decolorize a wide range of organic materials including organic dyes.

The work described in this communication was carried out as part of a project for the chemical and microbiological investigation of humus in soil, made possible by a grant from the Nuffield Foundation. Our thanks are also due to Dr. F. M. Dean for his help and advice on the chemical aspects of the work and to Miss D. S. Griffiths for technical assistance.

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[1] Flaig, W., and Schmidt, H. L., *Archiv Mikrobiol.*, **27**, 1 (1957).
[2] Schönwälder, H., *Archiv Mikrobiol.*, **30**, 162 (1958).
[3] Burges, A., and Latter, P., in "Symposium on Ecology of Soil Fungi" (Liverpool Univ. Press) (in the press).
[4] Evans, L. T., and Russell, E. W., *J. Soil Sci.*, **10**, 119 (1959).
[5] Boulter, D., and Burges, A., *Experientia*, **11**, 188 (1955).
[6] Nobles, M. K., *Can. J. Res.*, C, **26**, 281 (1948).
[7] Cartwright, K. St. G., and Findlay, W. P. K., "Decay of Timber and its Prevention", 179 (H.M. Stationery Office, 1946).
[8] Preston, A., and McLennan, E. I., *Ann. Bot.*, N.S., **12**, 53 (1948).

A Method for Characterizing *Pseudomonas solanacearum*

Pseudomonas solanacearum (E. F. Smith) is an important and widespread bacterial pathogen of economic plants in tropical and warm temperate zones[1]. It has been shown recently that the cells of *P. solanacearum* grown on an appropriate medium are intensively sudanophilic, due to the presence of massive inclusions of poly-β-hydroxybutyric acid[2,3]. Formation of the polymer is known to be a characteristic of certain saprophytic pseudomonads[2,4,5]. A further study was made to ascertain whether such a distinctive character could be diagnostic.

Plant pathogens from the genera *Xanthomonas*, *Pseudomonas*, *Agrobacterium*, *Erwinia*, and *Corynebacterium* were tested for production of a sudanophilic cell component by growing them on a peptone medium containing glucose, sucrose, or glycerol, then staining with sudan black *B*[6], followed by extraction of the dried cells in chloroform to confirm the presence of poly-β-hydroxybutyric acid[2]. The results are summarized in Table 1.

Table 1. Differentiation of Some Plant Pathogenic Bacteria on the Basis of Formation of Poly-β-hydroxybutyrate Inclusions

Bacteria	Host plants	No. of cultures examined	Cells containing prominent sudanophilic inclusions (poly-β-hydroxybutyric acid)
Pseudomonas	Numerous	63 strains (33 spp.)	–
*P. solanacearum**	Solanaceae Musaceae, etc.	43	+
P. caryophylli	Carnation (*Dianthus caryophylli*)	3	+
P. stizolobii	Clover (*Trifolium* spp.) and velvet bean (*Stizolobium deeringianum*)	2	+
P. marginata	*Gladiolus* spp.	4	+ (2/4)
P. viburni	*Viburnum* spp.	1	+
Erwinia†	Numerous	13 strains (4 spp.)	–
Corynebacterium‡	Numerous	13 strains (9 spp.)	–

* The collection of *P. solanacearum* included 22 strains from solanaceous hosts (9 from potato) and 9 strains from musaceous hosts.
† Including *E. carotovora* var. *atroseptica* (blackleg of potato).
‡ Including three strains of *C. sepedonicum* (ring rot in potato).
In addition, 44 strains from 18 species of *Xanthomonas* and 10 strains from 6 species of *Agrobacterium* were examined. Relatively small sudanophilic inclusions were found in five strains of *X. albilineans* and in one strain of *A. tumefaciens*. The concentration of poly-β-hydroxybutyric acid was about 1 per cent of the cell dry weight formed on 2 per cent sucrose/peptone medium. Sudanophilic inclusions were not observed in the other cultures of *Agrobacterium* or *Xanthomonas*.

The 43 cultures of *P. solanacearum* were of wide geographical distribution and included strains from Brazil, Central America, southern United States, Israel, Cyprus, Southern Rhodesia, Uganda, Kenya, and Mauritius, as well as isolates from seed potatoes imported in 1959 from Egypt into Scotland and Sweden. The morphology and consistency of colonies of *P. solanacearum* on solid medium are known to be correlated with virulence in solanaceous hosts[7]; this collection included those which were predominantly opaque and fluidal (virulent), and also those which were exclusively translucent and non-fluidal (avirulent or reduced virulence), but production of the polymer was characteristic of all strains of the pathogen. In stained preparations of fluidal and non-fluidal colonies grown on the sucrose/peptone medium defined below, it was observed that cells from the former colony type stained more uniformly with sudan black *B* than non-fluidal cells. Two strains of *P. solanacearum*, *F2* from tomato and *RE* from banana, showing in culture a clearly defined, apparently irreversible, change in colony morphology from fluidal (smooth) to non-fluidal (rough), were examined in order to correlate colony morphology and virulence with amount of poly-β-hydroxybutyric acid. The difference in intensity of staining with sudan black *B* was reflected in the quantity of polymer extracted from the cells (Table 2). Strain *RE* had previously been shown to be virulent in the fluidal form for banana and avirulent in the non-fluidal form. The same result was obtained for strain *F2* in tomato[8,9].

Table 2. Correlation between Colonial Morphology and Poly-β-hydroxybutyric Acid Content in *Pseudomonas solanacearum*

Strain	Colony type	Fluidal (smooth)			Non-fluidal (rough)		
	Age of cultures (hr.)	Cell dry weight (mgm./plate)	Polymer (mgm./plate)	Percentage polymer in cell (dry weight)	Cell dry weight (mgm./plate)	Polymer (mgm./plate)	Percentage polymer in cell (dry weight)
F2 (from tomato)	24	16·3	3·3	20·2	23·0	3·0	13·0
	48	40·0	14·3	35·7	29·5	8·5	28·8
	72	40·7	19·3	47·4	30·5	8·8	28·9
RE (from banana)	36	17·3	4·0	23·1	21·0	3·3	15·7
	60	32·8	13·0	39·6	28·3	7·5	26·5
	84	37·1	18·0	48·5	28·8	7·0	24·3

Poly-β-hydroxybutyric acid was extracted with chloroform from the acetone-dried cells obtained from the surface of three or four 20-ml. quantities of solid medium in 9 cm. diameter Petri dishes inoculated over the whole surface. Incubation temperature, 30° C. Concentration of sucrose in medium, about 20 mgm./ml. (see text for complete composition).

The following medium was found to be satisfactory for detection of poly-β-hydroxybutyric acid and observation of colony variants in *P. solanacearum* (amounts in gm./litre of distilled water): sucrose (B.D.H., Ltd., laboratory reagent), 20·0; peptone (Oxo, Ltd., London), 5·0; dipotassium hydrogen phosphate, 0·5; crystalline magnesium sulphate, 0·25; agar powder (Davis), 20·0, *p*H adjusted to 7·2–7·4 with 40 per cent sodium hydroxide, autoclaved at 15 lb. pressure for 20 min.

In systematic bacteriology detection of poly-β-hydroxybutyric acid has proved to be a valuable ancillary test; in plant pathology there may also be applications. The milky exudate from the infected vascular bundles of potato, tomato, and banana, from which almost pure cultures of *P. solanacearum* have since been obtained, has been shown to be intensely sudanophilic. The technique may prove useful, for example, in differentiating in plant tissue the three bacterial pathogens of potato: *P. solanacearum* causing brown rot, *Erwinia carotovora* var. *atroseptica* causing blackleg, and *Corynebacterium sepedonicum* causing ring rot, and possibly in the field diagnosis of other diseases.

Previous work has shown that poly-β-hydroxybutyrate inclusions are not formed in representative species of several genera of the Enterobacteriaceae, to which in the present work the genus *Erwinia* is added[9,10]. The absence of the polymer from all but one strain of one species of *Agrobacterium* contrasts with *Rhizobium*, in which massive inclusions are formed both in culture and in the bacteroids of root nodules[2,3].

I am grateful to Dr. W. J. Dowson, Botany School, Cambridge, for the opportunity to examine the bacteria maintained in his laboratory, and for his advice and encouragement. Many of the bacteria examined were obtained through the courtesy of Mr. R. A. Lelliott, curator of the National Collection of Plant Pathogenic Bacteria, Plant Pathology Laboratory, Harpenden.

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[1] Kelman, A., Tech. Bull. N.C. Agric. Exp. Sta., No. 99 (1953).
[2] Forsyth, W. G. C., Hayward, A. C., and Roberts, J. B., *Nature*, **182**, 800 (1958).
[3] Hayward, A. C., Forsyth, W. G. C., and Roberts, J. B., *J. Gen. Microbiol.*, **20**, 510 (1959).
[4] Morris, M. B., and Roberts, J. B., *Nature*, **183**, 1538 (1959).
[5] Doudoroff, M., and Stanier, R. Y., *Nature*, **183**, 1440 (1959).
[6] Burdon, K. L., *J. Bact.*, **52**, 665 (1946).
[7] Kelman, A., *Phytopath.*, **44**, 693 (1954).
[8] Spence, J. A., and Hayward, A. C. (unpublished work).
[9] Anon., *Rev. App. Mycol.*, **38**, 294 (1959).
[10] Hayward, A. C., *J. Gen. Microbiol.*, **21**, ii (1959).

True's Beaked Whale from the South-East Coast of South Africa

Mesoplodon mirus True, True's beaked whale, is known from fourteen stranded specimens from the shores of the North Atlantic, the most northerly specimen being from the Outer Hebrides and the most southerly from Florida[1]. On May 21, 1959, a 17 ft. 6 in. beaked whale was washed up at Wilderness, 170 miles eastwards along the coast from Cape Agulhas, the southernmost tip of Africa. The skeleton of this whale was saved from the axes of the health authority's disposal squad by Mr. B. L. Cairncross, a local naturalist, and presented to the South African Museum. This whale proves to be an adult male True's whale. Dr. F. C. Fraser has kindly compared photographs of this specimen with the example of True's whale in the British Museum (Natural History), and confirms this identification, stating, however, that the lower jaw of the South African specimen appears deeper and more massive than the British Museum example.

Moore and Wood[1] have regarded this species as primarily a western Atlantic species, occurring north of latitude 35° N., and consider the single Florida specimen (latitude 29° 28′ N.) reported by them as probably a stray carried southward of its normal range by the south-flowing current inshore of the Gulf Stream. The finding of the present specimen in the Indian Ocean greatly extends the range of the species, possibly indicating a southern variety. It also shows, however, that the beaked whales are so little known, particularly in areas where coast lines are very poorly covered for strandings as is the case over most of the Indian Ocean, that any generalizing as to distribution is really guesswork at present.

This specimen will be fully described and figured elsewhere.

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[1] Moore, J. C., and Wood, F. G., *American Mus. Novitates*, No. 1831, 1 (June 21, 1957).

A Bivalve Gastropod

In *Nature* of March 12, p. 749, Drs. L. R. Cox and W. J. Rees reviewed a paper by the Japanese authors Kawaguti and Baba on a bivalved gastropod they had described under the generic name *Tamanovalva*. I should like to put on record a further occurrence of this remarkable group of molluscs. A single left valve (now in the Stanford University Collection, California) of what is probably a new species, with the

characteristic spiral nucleus and central adductor muscle scar, was collected on Espiritu Santo Island, near La Paz, Gulf of California, in beach drift, on November 1, 1959.

The earliest generic name for this group of bivalved gastropods may prove to be *Berthelinia* Crosse, 1875 (*Journal de Conchyliologie*, vol. 23, p. 79), the type species of which, *B. elegans*, is from the Eocene of France. Specimens in the Paleontology Museum, University of California, have been available to me for study. I can see no significant differences in the shells between *Berthelinia* and the Recent forms, *Tamanovalva* from Japan and *Edenttellina* from Australia (until recently the latter had been assumed to belong to the Pelecypoda). That this opisthobranch gastropod group should have originated in the Paris Basin during Eocene time is not implausible, for many other molluscan groups now having a limited distribution in the Pacific Ocean flourished there during the early Tertiary. Whatever generic name is eventually accepted for these bivalved gastropods, the range of the group seems to extend back in time at least to mid-Eocene and in the modern seas from Japan to south-eastern Australia and to tropical West America.

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Developments in the Propagation of Clones of *Hevea brasiliensis* by Cuttings

Clones of *Hevea brasiliensis* are normally bud-grafted on seedling rootstocks. In an attempt to overcome the disadvantages inherent in this practice, the Rubber Research Institute of Malaya is conducting an investigation of methods of establishing clones as trees growing on their own roots. Encouraging results are being obtained by rooting leafy clonal cuttings under special conditions which include the application of water as a mist spray.

Two clones recommended for large-scale planting in Malaya, *PB* 86 and *GT* 1, are being rooted with up to 90 per cent success. Other less-widely distributed clones have rooted similarly, including some of the hybrids of *Hevea brasiliensis* and *H. benthamiana*. It is now possible to obtain sufficient clonal material to investigate the stock–scion relationships in *Hevea*, and to improve the accuracy of other botanical and nutritional experiments. Furthermore, should these self-rooted trees prove satisfactory in the plantation itself, the mist-spray propagation techniques will find large-scale commercial application—either in propagating high-yielding clones on their own roots, or in raising selected clonal rootstocks for panel- or crown-grafted clonal scions.

In common with many forest trees, cuttings from young seedlings of *Hevea brasiliensis* root readily, while those from buddings do not[1]. As the seedling ages so rooting ability declines, in rubber quite sharply[2]. Some progress in the production of uniform material was made by Wiersum[3], who by 'stumping' a young seedling (that is, by cutting it back very severely) and taking cuttings from the resulting sprouts obtained 40 rooted plants from one individual. McIndoe[4] also found that cuttings from the lower 3 ft. of seedling trees root readily and that this process can be repeated with the vegetative progeny. He hopes that this high-rooting capacity may persist, but the performance and secondary characteristics of such untested material are unknown and the method is limited to experimental use.

Levandowsky[5] first reported the rooting of commercial budded clones in some quantity by leafy cuttings. While still attached to the parent 'stump', each shoot was wounded by ringing the bark. Some days later these shoots were removed, together with the portion of callused tissue at their base, and inserted in the rooting medium under a continuous mist spray.

Experiments at this Institute designed to study the effect and interplay of the various factors have now shown that the ringing operation is unnecessary. A more important factor is the efficiency of atomization of the spray, accomplished by raising the pressure to 50 lb. per sq. in. and the use of special nozzles. The present procedure in propagating *Hevea* by leafy cuttings is as follows. Bud-wood stems are cut back to 30 in. above the graft union, and the shoots arising from the latent buds are used as cuttings. Shoots from the top of bud-wood stems have also rooted well, and with certain clones even leafy shoots from trees in tapping have been used with success. Cuttings are selected which have healthy, fully expanded and mature but not senescent leaves; the terminal bud must not be breaking into fresh growth. The source shoots are cut when about 15 in. long, the lower leaves removed and the shoots transported in polythene bags. In the propagation nursery their bases are trimmed to provide cuttings about 10 in. long. After immersion in water the coagulated latex at the cut end is peeled off and the basal inch dipped into 'Fermate' dust (kindly supplied by Messrs. E. I. Dupont de Nemours and Co., Inc.). The prepared cuttings are planted into beds 5 ft. wide containing a rooting medium, a 1 : 1 mixture by volume of sawdust and sand being suitable. Finely atomized water is applied through nozzles and switched off only at night or during rain. The beds are shaded to reduce the light intensity to about 50 per cent and are surrounded by a screen to prevent excessive drifting of the mist.

With some clones rooting begins after about 40 days, a number are less rapid in their rooting while in others there is a rapid deterioration of leaf condition accompanied by complete failure to root. When the roots are approximately 1 in. long the cuttings are planted into baskets containing John Innes Potting Compost No. 2 and placed at the sides of the rooting beds in the drift of the mist. After a further week the rooted plants are moved, and when sufficiently hardened and the roots well established, they are planted out.

Rooted cuttings of more than fifty clones have been produced and successfully planted, and their subsequent growth and rooting habit are being observed. An experiment to compare the growth of self-rooted clones with conventional bud-grafts on unselected seedlings stocks has been planted. Other related experiments are being planned.

Meanwhile attempts are being made to improve the rooting of other recommended clones. These include the production of source shoots in suitable condition for regenerative response, the use of growth regulating substances and other chemicals, and the determination of the optimal rooting environment.

Results of these studies will be published in due course.

We are obliged to Mr. C. E. T. Mann, director of the Rubber Research Institute of Malaya, for permission to publish this information and to Dr. F. R. Tubbs, director of the East Malling Research Station, for seconding one of us (R. J. G.) to the Rubber Research Institute of Malaya.

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[1] Baptiste, E. D. C., *J. Rubb. Res. Inst. Malaya*, **9**, 17 (1939).
[2] Gregory, L. E., *Turrialba*, **1**, 201 (1951).
[3] Wiersum, L. K., Rep. Fourteenth Int. Hort. Congr. Scheveningen, **2**, 1335 (1955).
[4] McIndoe, K. G., *Quart. Circ. Ceylon Rubb. Res. Sch.*, **34**, 39 (1958).
[5] Levandowsky, D. W., "Propagation of Clonal *Hevea brasiliensis* by Cuttings" (Agriculture Division, United States Operations Mission to Viet-Nam, Saigon, Nov. 1958).

ENTOMOLOGY

Wetting Properties of Insect Cuticle

The idea has long been current[1] that the tanning of insect cuticle by quinones makes it more 'hydrofuge'. It is certainly true that dry tanned gelatin is less easily wetted with water than the untanned protein. But it is doubtful if such a statement is meaningful, applied to a water-saturated protein, whether it be tanned or not. A more useful form of this original suggestion may be: the tanning of insect cuticle lowers its adhesion to water.

Recent experiments on the permeability of insect cuticle to water[2,3] suggest that the waterproofing is especially efficient when the waxes on the surface of the integument are packed in a solid monolayer, with the long aliphatic chains (*XY*, Fig. 1) pointing away from the cuticle surface, and with the polar endings (*YY*)—which are primarily hydroxyl groups—at the surface. The cuticle surface forming the substrate for such an orientated monolayer is not a simple protein, but a tanned lipoprotein[4]; it therefore now appears that the substrate has high adhesion to the hydrophilic, rather than to the aliphatic, ends of the polar molecules. Stated in this form, it would seem that tanned lipoprotein has marked hydrophilic affinities in circumstances where it might select either the hydrophilic or the lipophilic ends of polar molecules—the reverse of the thesis implied in the paragraph above.

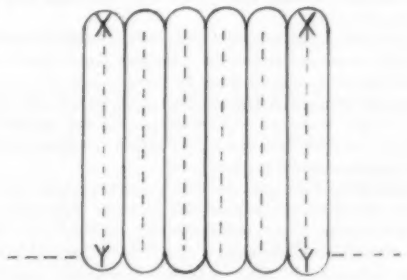

Fig. 1

Almost all substances, including solid paraffin wax, do become easier to wet after long exposure to water; but if the surface of a cockroach is washed in the surface of running water, whereby all its superficial unattached grease spreads away, the remaining surface is remarkably hydrofuge. From an assessment of the water-permeability, and of the permeability/temperature characteristics of such a washed animal, it would seem that only the orientated monolayer on its tanned cuticulin substrate remains behind[2], and that the extreme hydrofuge nature of this surface arises because such a monolayer will present an array of tightly packed —CH_3 groups (*XX*) to water placed on it—perhaps the surface with least adhesion for the water molecule. Recent experiments suggest that such a monolayer is likewise the structure over the surface of those hydrofuge areas of some aquatic insects, which can submerge for long periods, yet remain highly hydrofuge, as illustrated by their rapid shedding of water when they surface.

The evidence of recent studies on the permeability of these aquatic insects indicates, however, that they have an orientated monolayer of grease over their unwetted and over their readily wetted areas of surface as well. An examination of *Dytiscus* suggests that the cuticulin surface under the hydrofuge areas of grease is tanned, but that in the hydrophil areas of the natural cuticle, the underlying lipoprotein is not tanned. Now cuticulin and chorionin[5], which have large amounts of lipid condensed with protein, may well be expected to have a strong affinity for other lipoid materials, such as the aliphatic chains of polar greases—and much less affinity for hydroxyl groups. But the subsequent quinone tanning of a lipoprotein would appear to change this affinity, so that the surface then has high adhesion to the polar groups of the grease.

As a result of these observations and of physicochemical experiments, the following thesis can now be advanced: a change in the affinity of cuticulin or of chorionin may be promoted by tanning it with quinones; as a result a polarized grease layer on such a surface will be reversed, and consequently the surface property of a greased lipoprotein will change from being exceptionally hydrophil to being extremely hydrofuge, through the tanning of the substrate.

Since residual grease films are difficult to eliminate, such a phenomenon may give rise to the suggestion contained in the first paragraph, and attributed to the property change of protein alone. However, the implications of such a mechanism are much more profound. It may not only explain the way in which the aquatic insect may differentiate its surface properties; but also it may, for example, provide a real explanation for unusual capillary activities which must take place in all insects. Thus, if a tube or sponge is composed of a lipoprotein with a reversed monolayer on its surface, capillary pressures in it will be very high, for the contact angle to water against the outwardly directed polar groups will be very small. On tanning, and change of the monolayer to normal orientation, the contact angle will become appreciably greater than 90°; little energy will therefore be required to empty such spaces. Such phenomena occur in the emptying of the tracheal system at eclosion[6] and at ecdysis[7], in the production of the air film of the plastron while submerged in water, and in the appearance of air in the aeroscopic sponges of egg-shell[8]; the authors quoted have repeatedly outlined the necessity for assuming that a

mechanism is present which produces hydrofuge properties in a surface submerged in water, and have variously suggested that wax or its secretion, or the tanning of proteins, would prove essential to that unknown mechanism. The physico-chemical system now proposed would seem to satisfy these requirements and also to accommodate the associated evidence which has previously been described.

A full account of the experiments on which this suggestion is based, and of their probable implications for the physiological mechanisms of insects, will appear elsewhere.

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[1] Pryor, M. G. M., *Proc. Roy. Soc.*, B, **128**, 378 (1940).
[2] Beament, J. W. L., *J. Exp. Biol.*, **35**, 494 (1958).
[3] Beament, J. W. L., *J. Exp. Biol.*, **36**, 391 (1959).
[4] Wigglesworth, V. B., *Proc. Roy. Ent. Soc. Lond.*, B, **134**, 163 (1947).
[5] Beament, J. W. L., *Quart. J. Micro. Sci.*, **87**, 393 (1947).
[6] Sikes, E. K., and Wigglesworth, V. B., *Quart. J. Micro. Sci.*, **74**, 165 (1931).
[7] Wigglesworth, V. B., *J. Exp. Biol.*, **15**, 248 (1938).
[8] Wigglesworth, V. B., and Beament, J. W. L., *Quart. J. Micro. Sci.*, **91**, 429 (1950).

Effect of Relative Humidity on Hatching and on Toxicity of Ovicides to Eggs of the Red Spider Mite, *Tetranychus telarius* (L.) (Acarina : Tetranychidae)

Some information is already available on the relationship between relative humidity and hatching of eggs of *Tetranychus telarius* (L.)[1]. However, the information is based on data compiled from broad ranges of relative humidity and therefore does not enable an exact picture of the relationship to be seen.

In connexion with biological and toxicological studies in this laboratory on eggs of *T. telarius* it has been found convenient to induce mites to lay eggs on glass slides. This has made it possible to make an accurate study of the effects of relative humidity. By holding eggs in a particular relative humidity at 23° C. for the whole incubation period, the degree of resistance to desiccation or drowning over the whole range of relative humidities has been obtained. Results are given in Fig. 1.

With the red spider mite *Panonychus ulmi* (Koch), Beament[2] describes a change in the structure of the egg membrane which makes the egg 'waterproof' 6 hr. after it is laid. With *T. telarius* there appears to be no such complicating factor, the eggs having a certain resistance to desiccation and drowning from the moment of being laid.

Available information about the micro-climate on a leaf surface is unfortunately inconclusive. It can be assumed, however, that relative humidity can be high and in fact can reach saturation point. If such conditions of saturation or near saturation occurred for any prolonged period, it is clear that a high natural mortality of eggs could be expected.

Experiments with ovicides were carried out in relative humidities restricted to those which had little or no adverse effect on egg hatch. Freshly laid eggs of *T. telarius* were treated by dipping in ovicides (wettable powder formulations) and then incubated

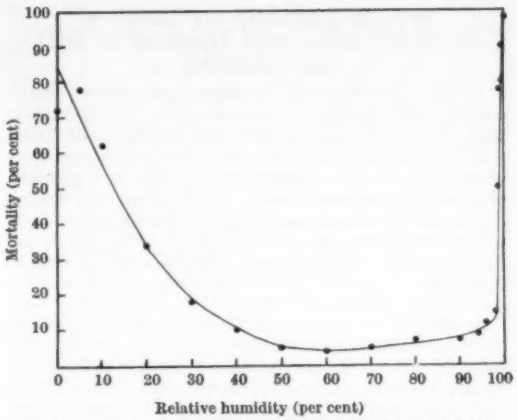

Fig. 1. Mortality of eggs of *T. telarius* in different relative humidities at 23° C.

in different humidities at 23° C. The concentration of ovicide which produced 50 per cent mortality of eggs (*LC*50 = lethal concentration 50) was determined at each humidity. Regression lines of mortality against concentration, obtained at each humidity, were parallel. Several ovicides were tested and all showed a similar trend : a decrease in *LC*50 with an increase in relative humidity. Results obtained for one of them, 'Kelthane' (1,1-*bis*(*p*-chlorophenyl)-2,2,2-trichloroethanol), are given in Fig. 2.

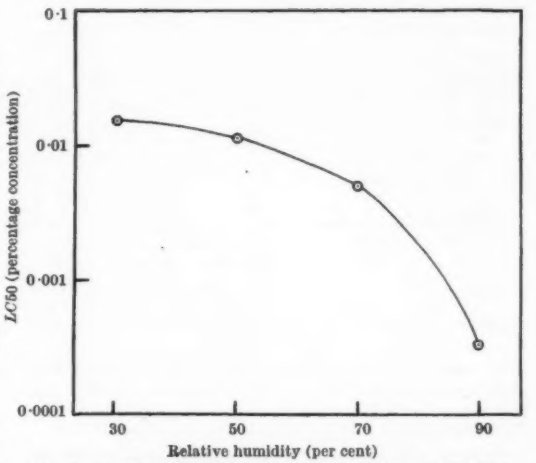

Fig. 2. Toxicity of 'Kelthane' to eggs of *T. telarius* incubated in different relative humidities. (An *LC*50 of 0·05 represents an application of ½ lb. active material per 100 gallons of water)

These results show that relative humidity must be considered as one of the significant factors affecting the performance of an ovicide.

A full report of this work is in preparation.

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[1] Boudreaux, H. B., *J. Insect Physiol.*, **2**, 65 (1958).
[2] Beament, J. W. L., *Ann. App. Biol.*, **38**, 1 (1951).

Normal Susceptibility Levels of *Anopheles pharoensis* Theobald to DDT and Dieldrin

House spraying with residual insecticides, particularly with DDT and dieldrin, plays an important part in modern anti-malaria campaigns. The tolerance of malaria vectors to these toxicants is therefore of special interest. Assessments of the susceptibility of many different species of anopheline mosquitoes before and during spraying operations have been carried out in many parts of the world[1]. However, nothing seems to have been published on the normal resistance-level of *A. pharoensis*, a vector of malaria in several countries of Africa.

A number of tests have been carried out on this species in the village of Natsini, 10 km. outside the sprayed area of the Mass Malaria Control Campaign in Sokoto Province, Northern Nigeria. Wild females were obtained at night from a donkey-baited hut soon after they had engorged. In the following morning they were exposed for 1 hr. to filter papers impregnated with insecticide using the World Health Organization adult test method, a modification of the Busvine test[2,3]. The exposure-time was followed by a recovery-period of 24 hr., during which the mosquitoes were kept in unwaxed paper cups provided with cotton wool soaked in sugar solution. Then the results were read.

The temperature and humidity during the time of testing varied from 24° C. to 28·4° C., and 74 to 85 per cent respectively, and the range of temperature during the recovery-period was 20–30° C. The results obtained are shown in Table 1.

Table 1

Insecticide	Concentration (per cent)	No. of mosquitoes exposed	dead	Mortality (per cent)
DDT	0·25	80	2	2
	0·5	240	30	12
	1·0	226	75	33
	2·0	307	218	71
	4·0	245	239	97
	4·0*	98	98	100
Control	—	163	0	0
Dieldrin	0·05	243	20	8
	0·1	288	100	35
	0·2	233	186	80
	0·4	227	223	98
Control	—	134	1	1

* Exposure time, 2 hr.

The median lethal concentrations (*LC*50) have been estimated graphically on the dosage-mortality lines drawn on logarithmic-probability paper. They were for DDT 1·5 and for dieldrin 0·14 per cent.

Susceptibility-levels, estimated for many different species of anopheline mosquitoes from untreated areas, have shown surprisingly little variation of the *LC*50[4]. It varies between 0·5 and 1·5 for DDT and between 0·05 and 0·15 per cent for dieldrin. The figures obtained in the present communication lie in the range of these variations and indicate a normal susceptibility of *A. pharoensis* to these insecticides.

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[1] World Health Organization, *Bull. WHO*, **16**, 874 (1957).
[2] Busvine, J. R., and Nash, R., *Bull. Entom. Res.*, **44**, 371 (1953).
[3] World Health Org., Tech. Report Series, **80**, 30 (1954).
[4] World Health Organization, WHO/Mal/203 (1958).

Mortality and Viability of Insect Migrants High in the Air

Entomologists have long been aware that vast numbers of insects are distributed up to thousands of feet in the air on any warm day[1]. The men who collected evidence of this assumed that the insects were alive[2–4]. However, the trapping methods they used killed most of the insects so that mortality and viability could not be assessed. Other entomologists have been less confident of the vitality of insects dispersing regularly by such flights, perhaps because of a belief that an ascent to, say, 5,000 ft. means a flight of many hours; this is not necessarily so[5]. Nevertheless, it is not generally accepted that this mode of transport is no more lethal than flight near the ground[6].

Insects have therefore been collected between 1,000 and 5,000 ft. at Cardington, Bedfordshire, England, with the kind co-operation of Mr. L. A. Speed and the staff of the Research and Development Establishment, to see what percentage of insects at these levels are alive and undamaged. The insects were caught in a vertical series of 6 nets, suspended from a balloon cable and opened for some hours between 09·00 and 16·00 hr. G.M.T. in June, July, August and September 1959. A mechanism opened the nets after ascent and closed them before descent[7].

Out of 1,610 insects caught, 1,571 (97 per cent) were alive and undamaged; 25 (2 per cent) were alive but damaged; 14 (1 per cent) were dead, when the nets were opened. Most of the damage to insects and some of the mortality were certainly caused by handling the nets. Mortality was probably also increased by the long time (up to 5 hr.) that some insects spent in the net after capture, exposed to the desiccating action of the wind. It may be assumed that the insects had, on average, completed half their aerial flight when they were caught, for the air almost clears of insects at night[1]. It can therefore be said with confidence that, of insects flying up to 1 mile high over southern England in mild summer weather, less than 2 per cent die in transit, except by predation or other means that leave no trace.

It is not possible to assess the viability of these insects quantitatively because it is often difficult or impossible to identify them at once and the pattern of reproductive behaviour is rarely known. However, some insects did reproduce, and living offspring were obtained from the following agricultural pests: cabbage aphid, *Brevicoryne brassicae* L.; peach-potato aphid, *Myzus persicae* Sulz.; bean aphid, *Aphis fabae* Scop.; frit fly, *Oscinella frit* L.; hessian fly group, *Mayetiola* sp., possibly *destructor* Say.

Many other insects, not yet identified, also reproduced successfully. Although not the smallest of insects, these include some of the most fragile. This seems to establish the viability of high-level migrants beyond reasonable doubt.

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[1] Johnson, C. G., *Sci. Prog. Twent. Cent.*, **39**, 41 (1951).
[2] Glick, P. A., Tech. Bull. U.S. Dep. Agric. No. 673 (1939).
[3] Hardy, A. C., and Milne, P. S., *J. Anim. Ecol.*, **7**, 199 (1938).
[4] Johnson, C. G., *J. Anim. Ecol.*, **26**, 479 (1957).
[5] Taylor, L. R., *Proc. Linn. Soc.*, **169**, 67 (1958).
[6] Andrewartha, H. G., and Birch, L. C., "The Distribution and Abundance of Animals", 109 (Chicago, 1954).
[7] Johnson, C. G., and Taylor, L. R., *Lab. Pract.*, **4**, 235 (1955).

GENETICS

Homeostasis of the Sex Ratio in *Drosophila*

THE ability of 150 larvæ to emerge as adults under 6 different temperature regimes for 3 inbred lines, their hybrids and the F_2 generation has been studied in *Drosophila melanogaster*[1]. This work revealed that interactions between genotype and environment were smallest in the F_1 generation and largest in the inbred lines. The F_1's therefore show a higher degree of stability or homeostasis than the inbred lines. The F_2 gave a genotype × environment interaction intermediate between the F_1 and the inbred lines, as might intuitively be expected because of segregation in the F_2.

The temperature regimes were 15° C., 24° C. for 8 hr. and 15° C. for 16 hr., 24° C., 24° C. for 8 hr. and 31° C. for 16 hr., 31° C., and 31° C. for 16 hr. and 15° C. for 8 hr. At 10 a.m. and 6 p.m., the replicates under the fluctuating temperatures were changed from one temperature to the other, so that the 16-hr. period represented the night and the 8-hr. period the day. The three inbred lines were Oregon (*OR*) and Sacramento (*SAC*), which were highly inbred by sib-mating, and Bikini (*BIK*), which has been mass-inbred since 1947. The *BIK* mass-inbred line was almost equivalent in homeostatic ability to the F_1 hybrids. This is perhaps because mass inbreeding permits the unconscious selection of heterozygotes more readily than under sib-mating.

While the data were being collected, sex ratios were noted throughout, and only recently have these been analysed. In Table 1, the percentage of females for each inbred line, F_1, and F_2, is given with a heterogeneity χ^2's testing the heterogeneity of the 2 × 6 contingency tables made up of the two sexes and the six temperature regimes.

Table 1. PERCENTAGE OF FEMALES AND HETEROGENEITY χ^2 TESTS

Inbreds	Females (per cent)	χ^2's for heterogeneity	Probability
OR	45·25	29·99	$P < 0.001$
SAC	48·97	9·42	$0.05 < P < 0.1$
BIK	50·41	2·15	$P > 0.9$
F_1's			
OR × *SAC*	51·88	9·00	$0.1 < P < 0.2$
SAC × *OR*	50·68	1·26	$P > 0.8$
BIK × *OR*	52·47	7·39	$0.1 < P < 0.2$
OR × *BIK*	51·80	3·50	$0.5 < P < 0.7$
BIK × *SAC*	51·91	5·62	$0.3 < P < 0.5$
SAC × *BIK*	52·38	1·18	$P > 0.9$
F_2's			
OR × *SAC*	52·51	12·55	$0.02 < P < 0.05$
SAC × *OR*	50·95	4·62	$0.3 < P < 0.5$
BIK × *OR*	51·37	11·29	$0.02 < P < 0.05$
OR × *BIK*	54·30	5·59	$0.3 < P < 0.5$
BIK × *SAC*	51·36	6·22	$0.2 < P < 0.3$
SAC × *BIK*	51·74	7·65	$0.1 < P < 0.2$

The *OR* and *SAC* inbred lines, which were inbred by sib-mating, were deficient in females and were relatively heterogeneous between temperatures, whereas the *BIK* mass inbred line gave 50·41 per cent females with no significant heterogeneity. Usually in *Drosophila* somewhat more females than males are expected[2], hence the *BIK* line is closer to the normal sex ratio than the *OR* and *SAC* lines. Furthermore, the *BIK* line showed no significant heterogeneity between temperatures, which again suggests its greater homeostatic ability compared with the *OR* and *SAC* lines.

All the F_1 crosses gave about 51 per cent females with no significant heterogeneity, thus demonstrating their superior homeostatic ability. The F_2's showed an excess of females, but with rather more heterogeneity than for the F_1 data, but less than the inbred lines. Similarly, as pointed out earlier, the smallest genotype × environmental interaction was obtained for the F_1's, the largest for the inbred lines, and the F_2 interaction occupied a position intermediate between the F_1 and the inbred lines. Hence it may be said that the sex ratio data in this experiment agree with the magnitude of the genotype × environmental interactions observed. The behaviour of the sex ratio in the various inbred lines and crosses over the six temperature regimes therefore agrees with recent theories on the possible relationship of homeostasis and heterozygosity[3]. It must of course be emphasized that homeostasis and heterozygosity are not necessarily related in all situations[4].

In order to explain the deficiency of females in the *OR* and *SAC* inbred lines, it may be postulated that the homozygous genes of the *XX* females depress the viability of the lines somewhat more than the hemizygous genes of the *XY* males. The F_1's between these inbred lines gave considerably more flies than the inbred lines[1], and as about 51 per cent females were produced in the F_1 compared with 46 per cent in the inbred lines, the increase is made up of rather more females than males. Possibly this occurs because the F_1 females are heterozygous for the *X* chromosome, whereas the F_1 males are hemizygous.

If heterozygosity is related to homeostasis or stability, as occurs in many situations[3], then it is possible that the *XX* females would exhibit a greater degree of homeostasis than the *XY* males. To test this, the significance of genotype × environmental interactions was tested for each sex separately for the inbred lines, and the F_1 and F_2 generations. However, there was no suggestion of any difference between sexes. Hence it may be said that within the limits of accuracy of the data, there is no difference in homeostatic ability between the sexes. Consequently the *X* chromosome may not be of great importance in determining homeostatic ability, unless the *Y* chromosome in the males has an effect similar to an *X* chromosome.

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[1] Parsons, P. A., *Genetics* (in the press).
[2] Crew, F. A. E., *Amer. Nat.*, **71**, 529 (1937).
[3] Lerner, I. M., "Genetic Homeostasis" (Oliver and Boyd, Edinburgh, 1954).
[4] Thoday, J. M., *Heredity*, **12**, 401 (1958).

Genetic Basis of Two Melanin Inhibitors in *Drosophila melanogaster*

MUTANTS such as ebony (*e*) and black (*b*) in *Drosophila* increase the melanin content of the integument of the adult fly. The ability of inbred and mass-bred larvæ of the genotypes $e^{ae}e^{ae}$, $e^{ae}+$, and $++$ to survive under various concentrations of the two melanin inhibitors phenyl-thio-carbamide and silver nitrate mixed in the food will be discussed briefly here, but complete details with additional information will be presented elsewhere.

Phenyl-thio-carbamide is an organic sulphur compound which inhibits melanin formation by combining with copper ions, which are necessary for the action of the enzyme, tyrosinase[1]. The inhibitory action of silver nitrate, a heavy-metal compound, is presumably due to its interaction with the enzyme

itself[1]. In the presence of sub-lethal doses of silver nitrate, flies are bleached to a yellow-white colour[2] and are often greatly reduced in size, but in the presence of sub-lethal doses of phenyl-thio-carbamide the flies are phenotypically unchanged except that some flies are slightly reduced in size.

Various concentrations of silver nitrate and phenyl-thio-carbamide were added to the medium on which larvæ not more than 12 hr. old were placed. The number of larvæ emerging as adults was recorded for mass bred $e^{e}e^{e}$, $e^{e}+$ and $++$ genotypes (e^{e} = ebony^{e}, $+$ = Oregon stock). Inbred lines of the same three genotypes were also tested. The inbred lines were produced by single pair mating individuals heterozygous for e^{e} for 17 consecutive generations. The ebony and wild-type homozygotes were then selected from the segregants of the seventeenth generation. Hence the three inbred lines should differ from each other genetically in the region of the ebony locus, but the remainder of the chromosomes ought to be almost identical genetically and nearly homozygous. In Table 1, some results are presented. Each entry in the table represents the number of adult flies emerging from 100 larvæ (4 replicates of 25 larvæ).

Table 1. Number of Adult Flies emerging from 100 Larvæ

Treatment	Mass bred			Inbred		
	$++$	$e^{e}+$	$e^{e}e^{e}$	$++$	$e^{e}+$	$e^{e}e^{e}$
Control	89	95	91	87	91	86
0·25 per cent $AgNO_3$	16	22	42	77	88	89
0·30 per cent $AgNO_3$	—	—	2	—	—	2
0·02 per cent P.T.C.	81	87	84	77	86	81
0·03 per cent P.T.C.	40	36	30	5	7	7
0·04 per cent P.T.C.	32	19	—	—	—	—
0·08 per cent P.T.C.	9	19	—	—	—	—
0·25 per cent $AgNO_3$ + 0·02 per cent P.T.C.	35	37	22	48	6	35
0·30 per cent $AgNO_3$ + 0·03 per cent P.T.C.	100	68	94	100	87	96

$AgNO_3$, silver nitrate; P.T.C., phenyl-thio-carbamide.

Considering first the mass-bred genotypes we see that silver nitrate is most lethal to $++$ flies and phenyl-thio-carbamide to $e^{e}e^{e}$ flies. The two inhibitors are therefore opposite in their action. Mixtures of the two chemicals in the medium might therefore lead to $e^{e}+$ flies emerging at higher concentrations than the two homozygotes. The table shows the results of two such tests. For 0·30 per cent silver nitrate + 0·03 per cent phenyl-thio-carbamide negative heterosis was observed. In fact, no mixture of silver nitrate and phenyl-thio-carbamide produced positive heterosis. Hence the two inhibitors appear to be complementary in their action on the heterozygote so that its viability is depressed most. Negative heterosis of this magnitude, where both the homozygotes survive, with very few or no heterozygotes, has evolutionary possibilities in the creation of isolating mechanisms. This situation suggests that by a suitable adjustment of the composition of the food medium, both $e^{e}e^{e}$ and $++$ could co-exist, but would be unable to exchange genes and hence they could diverge. Conditions are therefore created by which sympatric speciation can occur.

Another effect is noticeable at high concentrations of phenyl-thio-carbamide. At both 0·04 and 0·08 per cent phenyl-thio-carbamide, 19 $e^{e}+$ flies emerge. However, at 0·04 per cent, 32 $e^{e}e^{e}$ flies emerge and at 0·08 per cent, 9 emerge. Results not recorded here show that this trend is valid for intermediate phenyl-thio-carbamide concentrations. Hence we have a change in dominance according to concentration of phenyl-thio-carbamide. At 0·04 per cent, emergence appears to be correlated with the degree of melanization of the flies, but at 0·08 per cent there is no such correlation, and another system appears to have arisen which permits the development of heterosis.

Considerably more of the inbred flies survived on 0·25 per cent silver nitrate than mass-bred flies and silver nitrate is again most lethal to $++$ flies. This general increase in survival compared with the mass-bred flies is probably due to the inbreeding system used. As $e^{e}+$ is not much darker than $++$, it may be supposed that there would be a tendency for the darkest $e^{e}+$ flies to be selected so as to be sure they were $e^{e}+$. Such a selection did, in fact, appear to take place as the $++$ line extracted at the seventeenth generation of inbreeding was visibly darker than the $++$ mass-bred strain. Hence, as the flies were being inbred, selection was also being carried out simultaneously for increased melanin production. In subsequent generations, the melanin content of all genotypes would be increased, as a result of selecting dark $e^{e}+$ flies. In the mass-bred stocks, there was a correlation between melanin content and ability to emerge, and this relation appears to occur in the inbred stocks if it is assumed that selection has favoured more extreme melanization. Perhaps the melanins protect the developing fly by interacting with silver ions by a detoxification process, so that selection for increased pigment synthesis increases the tolerance of flies to silver ions with a resultant increase in survival value for all the inbred genotypes. With phenyl-thio-carbamide, therefore, we would expect fewer inbred flies to survive as there has been selection for increased melanization, for phenyl-thio-carbamide was most toxic to the mass-bred $e^{e}e^{e}$ flies. Table 1 shows that at 0·04 per cent phenyl-thio-carbamide no inbred flies emerged and at 0·03 per cent very few emerged. Hence, it is evident that the ebony locus is not the only locus affecting emergence under the two melanin inhibitors, but there is a substantial background of modifiers on which selection has acted. Finally, when silver nitrate and phenyl-thio-carbamide were mixed in the medium, negative heterosis was often observed. Many combinations did not show negative heterosis, but in no case did positive heterosis occur. Since inbreeding has only increased the general melanin-level, the occurrence of negative heterosis is to be expected in some cases.

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[2] Rapoport, J. A., *Amer. Nat.*, **81**, 30 (1947).

Basic Chromosome Numbers in *Silene* and *Saponaria*

During the course of a chromosome survey of the north-west Indian angiosperms, I have found two new basic numbers in the Caryophyllaceae of the Panjab Plains.

Silene conoidea Linn. is a very common annual weed in this area. The species has been worked out from several selections and has revealed persistently

10 bivalents at metaphase 1 with perfectly normal meiosis. In view of the fact that more than 80 species of the genus *Silene*[1] possess the basic number 12, the present number ($n = 10$) is significant. There is, however, one species, *S. fortunei*[2], with $n = 15$. These facts suggest that, first, the presence of the haploid numbers like $n = 10$ and 15 in the genus indicates that the original basic number of this series is 5. *S. conoidea* is, therefore, tetraploid and *S. fortunei* is hexaploid. Hence, both these species are what may be called 'cryptic polyploids'. Here it may be of interest to mention that the present observations reveal that the grade of ploidy built on $x = 5$ is from $4x$ to $6x$, while on $x = 12$ it is from $2x$ to $16x$[1].

Secondly, with the present observations the genus becomes dibasic ($x = 5$ and 12) and this at once raises the question about the probable constitution of $x = 12$, that is, whether it has been compounded from $x = 6$, or from $x = 5$ and 7. However, to date $x = 6$ and 7 have neither been discovered in any species of the genus, nor their presence surmised on even indirect grounds. In any event, the cytogenetical relationships between $n = 10$ in *S. conoidea* on one hand, and $n = 15$ in *S. fortunei* and species with $n = 12$ on the other, pose interesting problems. Furthermore, so far $x = 5$ has neither been found nor inferred on indirect grounds in any of the Silenoideae, but with the present observations one may be tempted to relegate $x = 15$, found in some genera of the subfamily, to only $x = 5$.

Lastly, in view of the overwhelming occurrence of $x = 12$ in the genus *Silene*, it is worthwhile to determine the biosystematical relationship of the species with $x = 5$, that is, whether the latter are genuine members of the genus.

Saponaria vaccaria Linn. is another very common annual weed. Several different samples of this species reveal the presence of $n = 15$ and $2n = 30$, which confirms the earlier findings of Mulligan[3]. Again this number is unique, since the prevailing situation in the genus is $n = 14$[1]. Apparently, this genus also becomes dibasic with $n = 14$ and 15, and it is worthwhile to find out the cytogenetical relationship between the two numbers. Such a study would also reveal the true taxonomical relationship of the present taxon with the typical species of the genus *Saponaria*. Another important aspect is the biosystematic relationship of *S. vaccaria* with the genus *Vaccaria*. Such a study is needed, particularly because, first, the present results reveal that the genus *Vaccaria* exclusively possesses $n = 15$, and secondly, *S. vaccaria* has been in the past often treated taxonomically as a member of the genus *Vaccaria*.

The above aspects are being studied in detail and I shall be obliged for viable seeds of *Silene fortunei* and any species of *Saponaria*, *Vaccaria* and their allied genera.

I am grateful to Prof. P. N. Mehra for granting facilities and for his interest in the study and to Mr. Baldev Raj for technical help.

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[1] Darlington, C. D., and Wylie, A. P., "Chromosome Atlas of Flowering Plants" (Allen and Unwin, Ltd., London, 1955). Cave, M. S., *et al.*, "Index to Plant Chromosome Numbers" (Calif. Bot. Soc., 1956, 1957 and Supplement); "Index to Plant Chromosome Numbers" (Univ. North Carolina Press, 1958).

[2] Heaslip, M. B., *Ohio J. Sci.*, **51**, 62 (1951).

[3] Mulligan, G. L., *Canad. J. Bot.*, **35**, 779 (1957).

PSYCHOLOGY

The Brain as Regulator

There has been some debate whether the brain is determinate or probabilistic in its behaviour. As a contribution to this question, the following argument shows that from one point of view the determinate system is demonstrably of greater efficiency than the probabilistic. The physiologist may therefore expect to find that natural selection has made the brain as determinate as possible.

If the brain is regarded as basically a means to survival, then a necessary condition for survival is that against the (moderately well-defined) set of disturbances that threaten the organism's existence the brain must so respond that the outcome of the combined action of disturbance and response keeps the organism's essential variables within normal limits[1-3]. The brain must, in other words, act as a regulator, homeostatic in the general sense. The sequence of disturbances that comes to the organism can then often be treated (algebraically at least) as an information source (in Shannon's sense[4]) having an entropy, $H(D)$ say. Similarly, the sequence of responses will have an entropy $H(R)$, and so will the sequence of values at the essential variables, $H(E)$. It is necessary for survival (though not sufficient) that $H(E)$ be kept small.

The most interesting case is that in which, if the organism does nothing, *all* the changes at D produce changes at E (that is, the organism is passively buffeted to full degree). In this case $H_R(E) = H_R(D)$, and it then follows[2] that $H(E)$'s minimum is given by $H(D) + H_D(R) - H(R)$. So far as $H_D(R)$ is concerned, the expression will be least if $H_D(R) = 0$. This relation gives a necessary condition that the brain must satisfy if it is to have maximal efficiency in generalized homeostasis.

The meaning of $H_D(R) = 0$ is readily specified. Of any source A, $H(A) = 0$ implies that the Markov chain must be such that when it reaches equilibrium the transitions that still occur are determinate (that is, have probabilities all 0 or 1). $H_D(R) = 0$ implies that this tendency to determinateness must hold for each value of D.

Thus, if the brain is survival-promoting by acts of regulation, it has maximal efficiency (other things being equal) if, under constant external conditions, it tends towards the deterministic way of behaving.

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[1] Ashby, W. Ross, "Design for a Brain", 2nd ed., 64 (Chapman and Hall, London, 1960).

[2] Ashby, W. Ross, "Introduction to Cybernetics", 197, 208 (Chapman and Hall, London, 1956).

[3] Sommerhoff, G., "Analytical Biology" (Oxford University Press, London, 1950).

[4] Shannon, C. E., and Weaver, W., "The Mathematical Theory of Communication" (University of Illinois Press, Urbana, 1949).

A Differential Test of Arts/Science Aptitude

The increasing pressure of academic specialization raises the question of whether we can discover a test of differential aptitude for advanced work in arts and science subjects respectively. In a preliminary study, the performance of a large number of Cambridge undergraduates on a test of high-grade intelligence (*A.H.5*) has been analysed, with special reference to relative ability on the verbal, numerical and diagram-

matic sections of the test[1,2]. Two differential scores were calculated as follows: 'Score *A*', diagrammatic minus numerical plus verbal; 'Score *B*', diagrammatic plus numerical minus verbal.

Two main results emerged. (*a*) If both differential scores were considered, a significant discrimination was found between arts and science students ($P = 0{\cdot}001$). (*b*) There were important differences in academic attainment between students with typical and atypical scores. The arts/science discrimination is shown in Table 1, where for reasons of convenience the distributions of scores have been divided into three groups: 'arts' scores, 'science' scores, and a 'neutral' group in which there was no pronounced discrimination. The group of arts students shown in Table 1 includes classicists, historians, linguists and students reading English. The group of science students includes mathematicians, physicists, engineers, botanists, zoologists and medical students. For reasons given below, it does not include students taking chemistry, biochemistry or metallurgy in Part 2 of the Tripos. With this one exception, students were classified in terms of the subject of the last Tripos taken, whether Part 1 or Part 2.

Table 1. Discrimination effected by *A.H.5* between Arts and Science Undergraduates at Cambridge

	'Arts' scores (per cent)	'Neutral' scores (per cent)	'Science' scores (per cent)
Arts students ($n = 164$)	66	28	6
Science students ($n = 411$)	7	45	48

From Table 1 it will be seen that the discrimination between arts and science students is very striking. The chances, for example, of a physicist having an 'arts' score, or a classicist having a 'science' score are as low as 1 in 20. The gist of the discrimination is as follows: arts students tend to score well on the verbal questions, and relatively poorly on the numerical and diagrammatic ones. The physical scientists (physicists, engineers, mathematicians) score well on the diagrammatic and numerical questions, and are relatively weak on the verbal ones. The biological scientists (botanists, zoologists and medical students) are relatively strong only on the diagrammatic questions. The only important exceptions are the students taking chemistry, biochemistry and metallurgy in Part 2 of the Tripos, who tend to be equally strong or weak on all three types of questions. Why this should be so is not at all clear.

Moreover, it was found that students with atypical scores (for example, a physicist with an 'arts' score) were significantly more likely than other students (*a*) to change subject in the middle of their undergraduate work, and (*b*) to gain a poor degree at the end of it. Further, those students with atypical scores who did change subject were four times as likely to choose a subject which was more rather than less concordant with their test results. (For all three differences, $P = 0{\cdot}001$.) On the strength of this evidence, we conclude that *A.H.5* can validly be used as a test of arts/science aptitude, if given to undergraduates. The question then arises: Can *A.H.5* validly be used at the age of sixteen, when the arts/science choice is crucial? Preliminary results suggest that such a use is feasible.

Samples of top-stream schoolboys have been tested at four schools, two public and two grammar. Each group tested was at a different stage of specialization, ranging from the highly specialized (some had never had a science lesson) to boys who had not specialized at all. It might be argued that the arts/science discrimination found among undergraduates was an effect of specialization rather than its underlying cause. If this were true, we should expect to find marked differences in the scatter of test scores both between schoolboys and undergraduates, and between specialized schoolboys and schoolboys who are unspecialized. Table 2 shows the standard deviations of both differential scores for five school samples, and for a representative sample of Cambridge undergraduates. It will be seen that the scatter of scores for all groups, unspecialized schoolboys and undergraduates alike, is remarkably similar. Further, Table 3 shows that the arts/science discrimination among partially specialized schoolboys is nearly as strong as that among a representative sample of undergraduates (including chemists, biochemists and metallurgists, and also the students reading geography, economics and law). Although promising, these results are no proper substitute for a follow-up study and should be viewed with caution.

Table 2. Effects of Academic Specialization on the Standard Deviation of Differential Scores in Six Different Samples

	Average age	*N*	'Score *A*'	'Score *B*'
School *A*	14·3	59	4·30	5·69
School *A*	17·1	58	3·83	5·77
School *B*	15·9	42	3·61	5·68
School *C*	16·0	50	4·29	5·18
School *D*	16·2	65	4·03	5·77
Cambridge	21·0	100	4·19	5·72

Table 3. Arts/Science Discrimination among Schoolboys compared with that among Cambridge Undergraduates

	'Arts' scores (per cent)	'Neutral' scores (per cent)	'Science' scores (per cent)
Schoolboys ($n = 212$)			
Arts	40	39	21
Science	10	38	52
Undergraduates ($n = 603$)			
Arts	55	33	12
Science	9	46	45

The help and encouragement of Dr. A. W. Heim and Prof. O. L. Zangwill are gratefully acknowledged.

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[1] Heim, A. W., *Brit. J. Psychol.*, **37**, 70 (1947).
[2] Heim, A. W., "Manual for the Group Test of High-Grade Intelligence *A.H.5*" (National Foundation for Educational Research, 1956).

Letter Structure of the English Language

Information regarding the sequential dependencies among letters in the English language is of interest not only in connexion with linguistics and cryptography, but also because of its value for communication theory and for the psychology of language[1,2].

Only two sets of such data have hitherto been published. One[3] is based on the King James version of the Bible, and the other[4] on an unspecified source. As the source may be an important variable, we have analysed two samples of modern English. The first represents written language and comprises all the words in the editorial columns of *The Times* newspaper

Table 1. Digram Frequency for Written English
Second Letter

Table 1. Digram Frequency for Written English
Second Letter

First Letter	Space	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	Total
Space	0	1,544	739	563	339	315	520	233	514	1,089	36	33	316	497	325	987	571	43	348	816	2,663	150	74	822	0	55	0	13,592
A	319	0	131	192	170	0	31	92	0	169	10	61	491	110	843	2	89	0	609	449	735	28	163	32	5	151	2	4,884
B	6	58	7	0	0	415	0	0	0	45	9	0	134	0	0	104	0	0	69	17	15	118	5	0	0	77	0	1,079
C	46	196	0	35	0	297	0	0	254	128	0	48	51	0	0	354	0	3	58	5	189	55	0	0	0	19	0	1,738
D	1,102	69	1	0	24	357	0	14	1	159	2	0	24	19	2	95	1	1	26	52	0	62	6	0	0	35	0	2,052
E	3,030	368	4	202	444	222	76	52	10	113	1	6	283	199	769	17	92	23	1,001	613	196	9	129	84	98	140	0	8,181
F	604	99	0	2	0	88	71	0	0	128	0	0	23	0	0	247	0	0	76	0	32	56	0	0	0	4	0	1,430
G	351	65	0	0	1	182	0	24	111	61	0	0	18	1	23	109	0	0	85	23	9	28	0	0	0	10	0	1,101
H	321	637	2	0	0	1,751	1	0	1	382	0	0	3	1	7	271	0	0	21	9	61	21	0	0	0	13	0	3,502
I	5	116	41	303	156	151	99	101	1	2	0	31	284	138	1,125	338	36	0	160	654	614	3	144	0	17	0	18	4,537
J	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	28	0	0	0	0	0	19	0	0	0	0	0	67
K	91	4	0	0	1	131	0	0	0	28	0	0	6	0	22	0	0	0	0	11	0	0	2	0	0	0	0	296
L	533	226	0	8	166	403	34	4	0	268	0	11	369	10	2	180	12	0	14	36	63	32	21	6	0	268	0	2,666
M	201	239	35	2	1	440	3	0	0	162	0	0	5	65	3	201	119	0	1	59	1	67	0	0	0	11	0	1,615
N	1,216	144	0	215	558	343	31	433	3	147	4	24	35	30	40	281	2	3	0	268	495	24	16	10	0	58	0	4,380
O	650	39	52	57	74	20	544	38	2	52	5	33	148	320	825	106	74	1	599	142	202	446	128	191	2	21	1	4,772
P	56	191	2	0	0	209	0	0	14	52	0	0	127	11	0	205	70	0	231	27	31	52	0	0	0	5	0	1,283
Q	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	0	0	0	0	0	75
R	706	284	12	26	96	1,011	4	43	9	306	0	39	59	88	105	312	23	0	38	211	189	49	39	6	0	116	0	3,771
S	1,741	90	0	30	2	440	9	0	140	259	0	8	34	29	2	172	92	1	3	171	571	154	0	9	0	19	0	3,976
T	1,525	240	0	9	0	504	3	1	2,171	562	0	0	70	24	6	583	0	0	208	177	95	110	0	44	0	86	0	6,418
U	6	52	50	83	20	74	4	65	0	49	0	0	181	58	222	5	53	0	218	187	229	0	2	0	1	3	0	1,562
V	0	44	0	0	0	534	0	0	0	122	0	0	0	0	0	22	0	0	1	0	0	2	0	0	0	2	0	727
W	156	163	1	0	0	190	0	0	269	220	0	2	3	0	52	135	0	0	4	13	1	0	0	0	0	0	0	1,209
X	9	7	0	8	0	12	0	0	0	18	0	0	0	0	0	1	44	0	0	0	23	1	0	0	0	0	0	123
Y	918	4	2	3	0	58	0	1	2	15	0	0	2	15	7	17	5	0	1	36	4	0	0	3	0	0	0	1,093
Z	0	5	0	0	0	14	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	21
																											Total	76,150

(1) The number of words in the sample is given by the space row total. (2) The number of letters in the sample is given by the grand total less the space total.

Table 2. Digram Frequency for Conversational English
Second Letter

First Letter	Space	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	Total
Space	0	1,809	807	617	684	195	410	431	1,085	1,875	87	218	387	732	437	835	296	49	260	1,301	2,253	135	112	1,218	0	883	0	17,116
A	366	0	153	88	166	0	66	70	10	121	0	101	451	87	1,055	1	79	0	394	367	650	64	221	54	2	292	1	4,859
B	12	57	2	0	0	372	0	0	0	46	1	0	66	0	0	194	0	0	51	7	7	221	1	0	0	29	0	1,066
C	5	255	0	6	0	140	0	0	168	36	0	87	29	0	0	325	0	0	25	1	100	35	0	0	0	6	1	1,189
D	1,463	111	8	1	20	202	8	6	0	169	0	2	21	8	125	389	0	0	34	30	0	11	13	1	0	30	0	2,652
E	2,975	391	5	103	333	337	46	20	5	45	1	10	406	104	449	33	73	0	1,060	506	271	0	130	41	50	95	0	7,479
F	406	74	0	0	0	86	46	0	0	86	0	0	12	0	0	183	0	0	72	1	60	45	0	0	0	0	0	1,071
G	609	36	0	0	0	187	0	10	237	68	0	0	20	1	4	253	0	0	30	26	1	12	0	0	0	0	0	1,494
H	444	866	2	0	11	1,616	1	0	1	484	0	0	3	3	0	323	0	0	22	0	201	19	0	0	0	44	0	4,040
I	664	29	20	109	237	112	134	130	0	1	0	93	254	266	1,102	51	16	0	92	402	723	0	105	0	3	0	0	4,543
J	0	1	0	0	0	3	0	0	0	1	0	0	0	0	0	13	0	0	0	0	0	77	0	0	0	0	0	95
K	290	0	0	0	0	238	6	0	0	78	0	0	3	1	162	0	0	0	0	49	2	0	0	7	0	2	0	838
L	723	126	2	2	282	290	33	0	0	220	0	37	721	12	0	166	20	0	3	37	28	28	4	44	0	235	0	3,013
M	330	167	22	0	1	520	2	0	0	112	0	0	0	42	1	176	28	0	5	26	0	101	0	0	0	82	0	1,615
N	861	36	1	79	691	330	15	655	5	139	4	145	39	0	23	476	3	0	0	77	668	14	5	1	1	119	1	4,388
O	1,049	10	13	65	101	40	273	13	143	71	0	82	120	276	687	290	83	0	486	76	293	1,443	54	350	1	21	0	6,042
P	128	63	0	0	0	178	0	0	15	37	0	0	80	0	0	113	81	0	85	47	12	32	0	0	0	15	0	886
Q	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	50
R	944	132	4	9	96	678	13	36	24	245	0	46	33	18	71	162	9	1	87	186	64	41	5	2	0	205	0	3,111
S	1,501	180	2	20	3	510	1	0	405	118	1	16	12	8	71	257	67	0	0	82	400	126	0	12	0	10	0	3,802
T	2,470	192	0	10	0	448	8	0	1,612	232	0	0	65	8	14	775	3	0	78	225	132	34	0	17	0	21	0	6,344
U	604	11	16	65	24	18	4	123	0	55	0	0	286	46	139	0	105	0	294	247	437	0	10	0	0	4	0	2,488
V	0	5	0	0	0	592	0	0	0	62	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	663
W	331	327	0	0	1	258	5	0	326	204	0	1	0	0	48	223	0	0	21	16	1	0	0	0	0	3	0	1,764
X	3	4	0	12	0	3	0	0	4	2	0	0	0	0	0	0	22	0	0	0	7	0	0	0	0	0	0	57
Y	938	7	9	3	2	125	0	0	0	36	1	0	5	3	0	800	1	0	12	93	43	0	3	17	0	0	0	2,098
Z	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	3
																											Total	82,766

for five successive days. The second sample, representing conversational language, is the text of the British Broadcasting Corporation's radio serial "Mrs. Dale's Diary", also for five successive days. In both cases names of persons and foreign place names were excluded.

The analysis was made by a National–Elliott 405 electronic computer at the G.P.O.'s London Electronic Agency for Pay and Statistics (*Leaps*). Two programmes have been written. One gives digram frequencies, counts being printed for all possible digrams; letter frequencies and a word count are obtained as by-products. The second programme gives trigram frequencies; the printed results give counts only for those trigrams occurring in the sample analysed.

Tables 1 and 2 show respectively the digram frequencies of the two samples. Copies of the trigram results are available from us. With the material now at hand, words can be generated which are either zero-, first-, second- or third-order approximations to either written or spoken English.

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[1] Shannon, C. E., and Weaver, W., "The Mathematical Theory of Communication" (Univ. of Illinois Press, Urbana, 1949).
[2] Miller, G. A., "Language and Communication" (McGraw-Hill, New York, 1951).
[3] Newman, E. B., and Gerstman, L. S., *J. Exp. Psychol.*, **44**, 114 (1952).
[4] Pratt, F., "Secret and Urgent, the Story of Codes and Ciphers" (Robert Hale, Ltd., London, 1939).

A Possible Fundamental in the Behaviour of Young Nidifugous Birds

The great output of papers over the past few years on the phenomenon of imprinting has recently been reviewed by Hess[1] and this has been brought up to date by Thorpe[2].

Although it seems that imprinting may not necessarily be irreversible, and in fact may not be very different from other types of learning[2,3], it is still convenient to define it as "the process whereby certain juvenile birds become psychologically fixed upon the parent during the first very few days after hatching". Analyses of processes in young nidifugous birds covered by this broad definition have shown that imprinting may occur in response to a parent-object moving away from the juvenile or to various other visual stimuli including 'retinal flicker'[1,4,6], or to various acoustic stimuli[4,5].

All such analyses, however, utilize juveniles hatched in incubators or by the normal female not under detailed observation, that is, the analyses begin after the first possible stimulus-response period, the time of actual hatching, is over. In other words, incubator-raised juveniles may be 'incomplete' with respect to an appetitive behaviour-consummatory act complex involving the parent bird, and normally hatched birds will be 'complete' in this respect. Such a difference in experimental material may well result in differences in response; an unknown variable may have been operative.

Results suggesting this have been obtained in the course of work on eider ducklings (*Somateria mollissima*) in the Canadian arctic during the summers of 1958[7] and 1959[8]. Twelve eider ducklings imprinted to myself in varying degrees have been raised naturally in the field to at least one week old. After this some were raised on prepared food, some 'escaped', and some died. Most of the observed behaviour patterns have been checked by comparison with wild eiders of the same age at the same time and place. On the authority of Haldane[9], 20 randomly selected juveniles are regarded as enough for ethological statistical validity.

One of the most significant behaviour patterns observed is one which occurs immediately upon hatching. I propose to call this the 'brooding reflex' because it seems to be the mechanism whereby the newly hatched duckling orientates itself to the position of optimum warmth and mechanical protection beneath the female. It involves an active search by the duckling for a feeling of enclosure around the head or part of the head. In this way the duckling investigates its surroundings, the appetitive behaviour being a search for a space into which it can thrust its bill or head. Thus, a duckling a few seconds after hatching on my hand would actively follow my other hand once I had held its head between finger and thumb. A state of rest, or even of sleep, could be released in the ducklings merely by providing this contact stimulus on two sides of the head or bill. A higher degree of enclosure was even more significant, as long as the bird was warm and could breathe and wriggle. All the ducklings in my care showed this response to a constant degree, but more significant perhaps, is the fact that I found that I could release a similar quiescence in wild juveniles. To date, this response has been positive in every nidifugous juvenile I have handled including the imprinted eiders and three imprinted red-breasted mergansers (*Mergus serrator*), and varying numbers of wild juveniles of eider, old-squaw (*Clangula hyemalis*), Canada goose (*Branta canadensis*), ringed plover (*Charadrius hiaticula*), and purple sandpiper (*Erolia maritima*).

It seems that the 'brooding reflex' may be of widespread occurrence within the Anatidae, and even among nidifugous birds in general. Whether it is regarded as a conditioning immediately preceding imprinting, or as an integral part of the full imprinting process, it would appear to be a fundamental behaviour activity which has previously been overlooked.

Detailed results of these studies will be published in due course. The work was carried out with the financial support of a Carnegie Arctic Research Scholarship and field grants from the Banting Fund of the Arctic Institute of North America, and the physical assistance of Mr. C. W. Nicol, all of which I gratefully acknowledge.

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[1] Hess, E. H., *Science*, **130**, 133 (1959).
[2] Thorpe, W. H., *Ibis*, **101**, 337 (1959).
[3] Thorpe, W. H., "Learning and Instinct in Animals", 116 (London, 1956).
[4] Klopfer, P. H., *Ecology*, **40**, 90 (1959).
[5] Fabricius, E., *Acta Zool. Fenn.*, **68**, 1 (1951).
[6] James, H., *Canad. J. Psychol.*, **13**, 59 (1959).
[7] Driver, P. M., *Arctic*, **11**, 191 (1958).
[8] Driver, P. M., Ph.D. thesis (in preparation).
[9] Spurway, H., and Haldane, J. B. S., *Behaviour*, **6**, 34 (1953).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, May 2

UNIVERSITY COLLEGE (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Prof. Z. M. Bacq (University of Liège): "The Enzymatic Inactivation of Adrenalin and Noradrenaline".*

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room (second floor) of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Prof. L. Pearce Williams: "Faraday and the Atomic Theory".

SOCIETY OF ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. C. E. Tharratt: "Problems in the Launching of Black Knight", illustrated by lantern slides and a film.

UNIVERSITY COLLEGE (in the Embryology Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Jorge Dias (University of Lisbon): "The Makonde People: Conflicts of Culture".* (Further lecture on May 5.)

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting followed by Dr. H. Baines: "The Use of Photography in Industry".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Sir Olaf Caroe, K.C.S.I., K.C.I.E.: "The Geography and Peoples of the Himalayan and Karakoram Frontiers".

Monday, May 2—Wednesday, May 4

INSTITUTE OF PHYSICS, NON-DESTRUCTIVE TESTING GROUP (joint meeting with the SOCIÉTÉ FRANÇAISE DE MÉTALLURGIE, at the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1)—Conference on "Physical and Structural Aspects of Non-Destructive Testing of Metals".

Tuesday, May 3

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Teaching and Learning Machines" opened by Mr. C. E. G. Bailey.

PLASTICS INSTITUTE (at the Wellcome Building, 183–193 Euston Road, London, N.W.1), at 6.30 p.m.—Annual General Meeting, followed by a speaker from the Plastics Industry of the U.S.S.R.

ANTIQUARIAN HOROLOGICAL SOCIETY (at the Science Museum, South Kensington, London, S.W.7), at 7 p.m.—Annual General Meeting, followed by an illustrated talk on Mr. B. Hawkins' collection.

Tuesday, May 3—Wednesday, May 4

SOCIETY FOR ANALYTICAL CHEMISTRY (in conjunction with the IRON AND STEEL INSTITUTE and the INSTITUTE OF METALS, at Denison House, 296 Vauxhall Bridge Road, London, S.W.1)—Symposium on "The Determination of Gases in Metals".

Tuesday, May 3—Thursday, May 5

IRON AND STEEL INSTITUTE (at the Royal Commonwealth Society, Caxton Hall, and Church House, London)—Annual General Meeting, 1960.

Wednesday, May 4

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Dr. Ragnor Fjortoft (Oslo): "The General Circulation of the Atmosphere" (Symons Memorial Lecture).

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. Philip Chantler: "Europe's Energetic Forecasting".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. L. Pearce Williams: "Michael Faraday and the Evolution of the Concept of Electric and Magnetic Fields".

Thursday, May 5

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. S. Ainsworth, Mr. Q. H. Gibson and Prof. F. J. W. Roughton, F.R.S.: "The Kinetics of the Reactions of Sheep Hæmoglobin with *Iso*cyanides"; Mr. R. H. Dadd: "Some Effects of Dietary Ascorbic Acid on Locusts".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. H. R. Hewer: "Expedition to North Rona, October 1959"; Mr. N. W. Simmonds: "Useful and Ornamental—a Miscellany of Tropical Plants".

INSTITUTE OF REFRIGERATION (joint meeting with the INSTITUTION OF HEATING AND VENTILATING ENGINEERS, at the Institute of Marine Engineers, The Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. B. A. Phillimore and Mr. S. J. Jones: "The Demand for Cooling in the Modern Passenger Liner".

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. S. J. Emerson: Annual Lecture on "Safety in the Utilization of Electricity".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 5.30 p.m.—Annual General Meeting.

CHEMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 7.30 p.m.—Prof. A. Butenandt: Adolf Windaus Memorial Lecture.

Friday, May 6

INSTITUTION OF ELECTRICAL ENGINEERS, MEDICAL ELECTRONICS DISCUSSION GROUP (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Methods of Recording Measurements—Digital or Analogue" opened by Mr. W. J. Perkins.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH BIOPHYSICIST (preferably with experience of protein structural studies) IN THE IMMUNOLOGY DEPARTMENT, to investigate the structure and interactions of antibodies and antigens—The Secretary, St. Mary's Hospital Medical School (University of London), Paddington, London, W.2 (May 4).

SENIOR LECTURER (graduate, with experience in either personnel management, industrial communications, or teacher training) IN PSYCHOLOGY—The Registrar, Royal Technical College, Salford 5 (May 6).

READER IN GEOPHYSICS—The Secretary, The University, Exeter (May 7).

SENIOR LECTURER IN SOLID STATE PHYSICS—The Secretary, The University, Exeter (May 7).

SENIOR LECTURER (with high qualifications and wide experience) IN PHYSICS, to teach to honours degree level; and an ASSISTANT (Grade B) IN PHYSICS—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (May 7).

SENIOR RESEARCH STUDENT IN EITHER ENGINEERING OR CHEMICAL ENGINEERING OR METALLURGY—The Registrar, University College, Singleton Park, Swansea (May 7).

LECTURER or ASSISTANT LECTURER (with an honours degree in philosophy) IN PHILOSOPHY—The Registrar, University of Malaya in Kuala Lumpur, Pantai Valley, Kuala Lumpur (May 10).

ASSISTANT IN THE DEPARTMENT OF ZOOLOGY—The Secretary, The University, Edinburgh (May 14).

LECTURER (Grade II) IN PHYSICS; a RESEARCH ASSOCIATE IN LOW TEMPERATURE PHYSICS; and a RESEARCH ASSOCIATE IN THEORETICAL X-RAY CRYSTALLOGRAPHY in the H. H. Wills Physical Laboratory—The Registrar, The University, Bristol 8 (May 14).

LECTURER (with special qualifications in physiological psychology) IN PSYCHOLOGY—The Registrar, The University, Sheffield (May 14).

SENIOR LECTURER or LECTURER IN MATHEMATICAL LOGIC—The Registrar, The University, Manchester 13 (May 14).

STAFF TUTOR IN SCIENCE (PHYSICAL SCIENTIST)—The Registrar, The University, Sheffield 10 (May 14).

TEMPORARY ASSISTANT LECTURER or LECTURER IN SOCIOLOGY OR SOCIAL ANTHROPOLOGY—The Registrar, The University, Hull (May 14).

DEMONSTRATOR IN PHYSICAL CHEMISTRY—Head of the Physical Chemistry Laboratory, The University, South Parks Road, Oxford (May 15).

FELLOW IN PHYSICAL CHEMISTRY—St. Catharine's College Planning Office, 42 Banbury Road, Oxford (May 15).

LECTURER and an ASSISTANT LECTURER (with research experience in pure mathematics and preferably with qualifications in analysis) IN MATHEMATICS—The Registrar, University College of North Wales, Bangor, North Wales (May 16).

LECTURER (Grade II) IN PURE MATHEMATICS—The Registrar, The University, Bristol 8 (May 16).

LECTURER IN APPLIED MATHEMATICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (May 16).

REGIUS CHAIR OF MORAL PHILOSOPHY in the University of Aberdeen—The Private Secretary, Secretary of State for Scotland, St. Andrew's House, Edinburgh 1 (May 20).

LECTURERS (3) and ASSISTANT LECTURERS (3) IN THE DEPARTMENT OF CHEMISTRY—The Registrar, The University, Sheffield (May 21).

LECTURER IN PHYSIOLOGY—The Secretary, The University, Aberdeen (May 23).

ASSISTANT LECTURER IN THE DEPARTMENT OF APPLIED MATHEMATICS AND THEORETICAL PHYSICS—Secretary of the Appointments Committee of the Faculty of Mathematics, The Arts School, Bene't Street, Cambridge (May 25).

STUDENT DEMONSTRATOR (graduate) IN THE DEPARTMENT OF BOTANY, University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 25).

ASSISTANT LECTURERS (2) IN ZOOLOGY—The Registrar (Room 22, O.R.B.), The University, Reading (May 28).

LECTURER or ASSISTANT LECTURER (with special experience in food microbiology) IN THE DEPARTMENT OF MICROBIOLOGY—The Registrar (Room 22, O.R.B.), The University, Reading (May 28).

SENIOR ASSISTANT LIBRARIAN (suitably qualified academically and professionally), to take charge of the Library of the Faculties of Agriculture and Engineering, University College of the West Indies, Trinidad—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 30).

CHAIR OF MATHEMATICS in the University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, May 31).

LECTURER or SENIOR LECTURER IN GENETICS AND PLANT BREEDING; an ASSISTANT LECTURER IN BOTANY; and a DEMONSTRATOR IN ZOOLOGY—The Registrar, Wye College (University of London), near Ashford, Kent (May 31).

LECTURER (science graduate, with experience in bacteriology, immunology or virology, and in research methods) IN MICROBIOLOGY at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, May 31).

PROFESSOR OF MATHEMATICS OR APPLIED MATHEMATICS at the University of Natal, Durban, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, May 31).

RESEARCH FELLOW (with a Ph.D. degree or equivalent experience) IN THE CHEMISTRY DEPARTMENT, for fundamental work on compounds potentially useful in the paint industry—The Professor of Chemistry, The University, Hull (May 31).

SENIOR RESEARCH FELLOW (graduate in agricultural chemistry, biochemistry or physiology, with some experience of research in animal nutrition) IN POULTRY NUTRITION—The Secretary, University of Nottingham School of Agriculture, Sutton Bonington, near Loughborough (June 1).

EXECUTIVE ASSISTANT (with experience in administration and control of non-academic staff, and in writing and editing) IN THE SCHOOL OF PHYSICS, University of Sydney, to assist the Head of the School in the administration of the School and the Nuclear Research Foundation, and to edit and write the major portion of the Foundation's publication *The Nucleus*—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1, and the Registrar, University of Sydney, Sydney, N.S.W., Australia (June 3).

LECTURER/SENIOR LECTURER IN BIOCHEMISTRY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 3).

LECTURER IN THE DEPARTMENT OF PHYSICS—Prof. P. M. S. Blackett, F.R.S., Physics Department, Imperial College of Science and Technology, Imperial Institute Road, London, S.W.7 (June 14).

LECTURER or SENIOR LECTURER IN PSYCHOLOGY at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, June 15).

EDWIN CUTHBERT HALL CHAIR OF MIDDLE EASTERN ARCHÆOLOGY in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 30).

SENIOR LECTURER or LECTURER IN APPLIED CHEMISTRY at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, June 30).

EXPERIMENTAL OFFICER (at least 28, with a degree in engineering, H.N.C. or equivalent qualifications, and experience of research work) at the National Institute of Agricultural Engineering, to work in co-operation with the Department of Hop Research, Wye College (University of London) on engineering problems connected with the mechanization of hops and hop drying—The Secretary, National Institute of Agricultural Engineering, Silsoe, Beds.

LECTURER IN MATHEMATICS—The Registrar, Bradford Institute of Technology, Bradford 7.

RESEARCH ASSISTANT (with a first- or second-class honours degree in biochemistry), to assist with investigations into disturbances of calcium metabolism—The Director, Metabolic Disturbances in Surgery Research Unit, The General Infirmary, Leeds.

SENIOR LECTURER or LECTURER IN BACTERIOLOGY AND PARASITOLOGY at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

SOLID STATE SCIENTISTS, Scientific Officers (with at least a first- or good second-class honours degree and preferably with post-graduate experience in solid state studies), IN THE METALLURGY DIVISION, to study the basic mechanisms giving rise to the physical properties of certain nuclear ceramics in both single crystal and polycrystalline form—The Group Recruitment Officer (1639/34), U.K.A.E.A., Atomic Energy Research Establishment, Harwell, Didcot, Berks.

REPORTS and other PUBLICATIONS

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Great Britain and Ireland

Field Studies Council. Annual Report, 1958–1959. Pp. 34+4 plates (London: Field Studies Council, 1960.) [172

Choice of Careers No. 81: Forestry. Second edition. Pp. 36. (London: H.M. Stationery Office, 1959.) 1*s.* 9*d.* net. [172

Building Research Station Digest No. 130 (January, 1960): Supersulphate Cement. The Cracking of Asbestos-Cement Sheets. Pp. 4. (London: H.M. Stationery Office, 1960.) 4*d.* [172

Department of Scientific and Industrial Research. Fuel Research 1958: Report of the Director of Fuel Research for 1958. Pp. iv+35. (London: H.M. Stationery Office, 1959.) 2*s.* 6*d.* net. [172

Ministry of Agriculture, Fisheries and Food. Bulletin No. 174: Poultry Nutrition. By Dr. W. Bolton. Pp. v+90. (London: H.M. Stationery Office, 1959.) 5*s.* net. [172

Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series II, Vol. XXII, No. 9: Echo Sounding Experiments in the Barents Sea. By I. D. Richardson, D. H. Cushing, F. R. Harden Jones, R. J. H. Beverton and R. W. Blacker. Pp. vi+57. (London: H.M. Stationery Office, 1959.) 20*s.* net. [172

Cement and Concrete Association. Concrete Facing Slabs. By J. Gilchrist Wilson. Third (revised) edition. Pp. 30. (London: Cement and Concrete Association, 1959.) [172

Forestry Commission. Forest Record No. 39: Collection of Cones from Standing Trees. By D. T. Seal. Pp. 33. (London: H.M. Stationery Office, 1959.) 3*s.* 6*d.* [172

Newmarket Transistors Limited. Application Notes. Pp. 120. (Newmarket: Newmarket Transistors Limited, 1960.) [172

University of Birmingham, Report of the Vice-Chancellor and Principal for the Calendar Year 1959. Pp. 28. Report of the Proceedings of the Council of the University for the year ended 31st December, 1959. Pp. 18+4 plates. Accounts for the year ended 31st July, 1959. Pp. 35. University Research Committee Thirtieth Annual Report (Session 1958–1959). Pp. 123. (Birmingham: The University, 1960.) [172

University of Leeds. Annual Report of the Librarian, Session 1958–1959. Pp. 12. (Leeds: The Brotherton Library, The University, 1959.) [172

Other Countries

Publikationer fra det Danske Meteorologiske Institut. Communications Magnétiques, etc. No. 24: Local Aurorae in the Morning Hours at Godhavn. By K. Lassen. (A paper presented at the Auroral Conference in Uppsala, 25–26 August 1958.) Pp. 15. (Charlottenlund: Danske Meteorologiske Institut, 1959.) [172

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Osservatorio Astrofisico di Arcetro. Annuario per il 1960. Pp. 61. (Firenze: Coi Tipi dell'Istituto Geografico Militare, 1959.) [172

Seventh Annual Report of the West African Institute for Oil Palm Research, 1958–1959. Pp. 140. (Benin City: West African Institute for Oil Palm Research; London: Crown Agents for Oversea Governments and Administrations, 1959.) 2*s.* 6*d.* [172

Anuario del Observatorio Astronómico de Madrid para 1960. Pp. 406. (Madrid: Observatorio Astronómico, 1960.) [172

Australian Atomic Energy Commission Research Establishment. Pp.12. (Sydney: Australian Atomic Energy Commission, 1960.) [172

United States Department of Commerce: National Bureau of Standards. Applied Mathematics Series, No. 57: Basic Theorems in Matrix Theory. By Marvin Marcus. Pp. iv+27. (Washington, D.C.: Government Printing Office, 1960.) 15 cents. [172

United States Department of Commerce: Coast and Geodetic Survey. Publication 62–2: Geodimeter Manual. By Austin C. Poling. Pp. vi+70. (Washington, D.C.: Government Printing Office, 1959.) 45 cents. [172

International Atomic Energy Agency, Vienna. *Atomic Energy*, No. 6. (A Chronological List of Conferences, Meetings, and Training Courses.) Pp. 25. (Vienna: International Atomic Energy Agency, 1960.) [172

British Guiana: Geological Survey. 5th Inter-Guiana Geological Conference—Addresses to the Opening Session. Pp. 11. (Georgetown: Geological Survey, 1959.) [172

Institut de France. Académie des Sciences Annuaire pour 1960. Pp. 251. (Paris: Gauthier-Villars, 1960.) [172

Institut Royal des Sciences Naturelles de Belgique. Expédition Océanographique Belge dans les Eaux Côtières Africaines de l'Atlantique Sud (1948–1949). Résultats Scientifiques. Vol. 4, Fascicule 3B: Poissons. V: Téléostéens Acanthoptérygiens (Deuxième partie). Par Max Poll. Pp. 417+7 planches. (Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1959.) [172

United States Department of the Interior: Geological Survey. Bulletin 1028–L: Geology of Umnak and Bogoslof Islands, Aleutian Islands, Alaska. By F. M. Byers. Pp. viii+267–369+plates 39–51. Bulletin 1028–M: Geology of Southern Adak Island and Kagalaska Island, Alaska. By George D. Fraser and George L. Snyder. Pp. vi+408+plates 52 and 53. Bulletin 1028–N: Some Effects of Recent Volcanic Ash Falls, with special reference to Alaska. By Ray E. Wilcox. Pp. vi+409–476+plates 54–58. 30 cents. Bulletin 1069: Geology of the Thomas Range Fluorspar District, Juab County, Utah. By M. H. Staatz and F. W. Osterwald. Pp. v+97+12 plates. Bulletin 1072–G: Coal Resources of Trinidad–Aguilar Area, Las Animas and Huerfano Counties, Colorado. By R. L. Harbour and G. H. Dixon. Pp. iv+489+plates 10–14. Bulletin 1079: Geology of the Charleston Phosphate Area, South Carolina. By Harold E. Maide. Pp. v+105+10 plates. 1.75 dollars. Bulletin 1082–C: Iron-Ore Resources of the United States including Alaska and Puerto Rico, 1955. By Martha S. Carr and Carl E. Dutton. Pp. iv+134+plate 2. 75 cents. Bulletin 1087–A: Geology of the Garo Uranium-Vanadium Copper Deposit, Park County, Colorado. By V. R. Wilmarth. Pp. iii+21+5 plates. Bulletin 1087–C: Uranium Resources of the Green River and Henry Mountains Districts, Utah—a Regional Synthesis. By Henry S. Johnson, Jr. Pp. iv+59–104+plates 6–9. 1 dollar. Bulletin 1087–E: Stratigraphy and Uranium Content of the Chattanooga Shale in North-eastern Alabama, Northwestern Georgia and Eastern Tennessee. By Lynn Glover. Pp. iv+133–168+plates 14–18. 1 dollar. Bulletin 1097–A: Evaluation of the Lead-Alpha (Larsen) Method for Determining Ages of Igneous Rocks. By David Gottfried, Howard W. Jaffe and Frank E. Senftle. Pp. iv+63+plate 1. 35 cents. Bulletin 1106–C: Geophysical Abstracts, No. 178 (July–September, 1959). By Dorothy B. Vitaliano, S. T. Vesselowsky and others. Pp. iii+261–406. 40 cents. (Washington, D.C.: Government Printing Office, 1959.) [192

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